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PHYTOPATHOLOGY

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OBSERVATIONS ON FOREST PATHOLOGY IN GREAT BRITAIN AND DENMARK

J. S. BOYCE¹

INTRODUCTION

In the past, Great Britain has paid little attention to growing forests as compared with other European nations. Timber needed was readily and cheaply imported from the Continent and from America, so a "laissez faire" policy was pursued. During the World War, however, the country was in desperate need of timber supplies for use at the front and in the service of supply. Space in ships needed for carrying troops and munitions had to be given over to rough lumber. On the other hand, the Central Powers had been able to maintain themselves effectively from their adequate and well-managed woodlands. At the conclusion of the war Great Britain found it essential to begin extensive reforestation and afforestation. Her woefully inadequate supply of timber, particularly softwoods, was almost completely exhausted. Furthermore, there were large areas of almost waste land in the highlands of Scotland, on which the population was steadily decreasing. Forests on such land would give employment and increase the population.

Unfortunately there is only one conifer of commercial value native to Great Britain, that is, Scotch pine (*Pinus sylvestris* L.), and even its nativ-

¹ During the period May 29 to October 7, 1925, the writer was in Great Britain for the purpose of studying forest tree diseases in general and Douglas fir canker in particular. One short trip was made to Denmark. Throughout the course of this work the writer was accorded every possible assistance. Particular acknowledgement is made to Dr. Malcolm Wilson, Consulting Mycologist to the Forestry Commission, who generously placed his laboratory and his extensive information on tree diseases in Great Britain at the disposal of the writer. Thanks are also due Dr. A. W. Borthwick, Prof. Fraser Story, Dr. H. M. Stevens, Dr. Mark Anderson, and Mr. J. S. L. Waldie of the Forestry Commission for many courtesies, to Dr. Augustine Henry for information on coniferous species, to the Royal Scottish and the Royal English Arboricultural Societies for the privilege of attending their field excursions, and, finally, to Skovrider K. Bramsen in charge of the state forest at Almindingen on the island of Bornholm in Denmark for making possible observations there.

ity is subject to dispute. This species, together with European larch (*Larix europaea* D.C.), introduced so long ago that many believe it to be native, comprised the bulk of the softwoods grown. These two trees grow slowly, however, and fast-growing trees were needed. Furthermore, they are quite unsuitable to certain sites which cover extensive areas.

While in the past the British, with some exceptions, have been little interested in forestry, they have been enthusiastic arboriculturists, so that specimens of conifers of considerable age from all over the world may be found on the island. These showed that species from that portion of the Pacific Coast of North America north of the California line and west of the summit of the Cascade Mountains were particularly suitable for culture. In fact certain of these species in Britain grow more rapidly, in their youth at least, than they do in their native home. This is probably explainable by climatic differences. In England and Scotland the summers are normally mild and moist—moderate temperatures, abundant showers, and high humidity, while at home during the same period the trees are subjected to relatively high temperatures and long periods of drouth. Mild, wet winters are the rule in both regions.

Of the conifers from the Pacific Coast, Douglas fir (*Pseudotsuga taxifolia* (Lam.) Br.) is most extensively planted, followed by Sitka spruce (*Picea sitchensis* (Bong.) Trautv. and Meyer). Grand fir (*Abies grandis* Lind.) has found considerable favor. Western red cedar (*Thuja plicata* Don.), Port Orford cedar (*Chamaecyparis lawsoniana* Parl.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) are used to some extent. Noble fir (*Abies nobilis* Lind.) is being tried in a small way. Japanese larch (*Larix leptolepis* Murr.) is the only oriental species commonly planted. The continental European species most used are Norway spruce (*Picea excelsa* Lk.) and European larch. Silver fir (*Abies pectinata* D.C.) has rapidly declined in favor, owing to the ravages of the silver fir aphid (*Dreyfusia nuesslini* Börner), presumably imported from the Continent, where it is common on the same host but doing much less damage. Among the pines, Scotch pine is still most fancied, but maritime pine (*Pinus pinaster* Sol.) and Corsican pine (*P. laricio* Poir.), both native to southern Europe, are extensively used. Lodgepole pine (*P. contorta* Loud.) from the northern Rocky Mountains is being experimented with.

But as far as private owners are concerned, the species which is playing a very large part in the planting campaign is Douglas fir, on account of its phenomenal rate of growth. This tree, first introduced into England in 1827 (9, p. 35), almost 100 years ago, was not planted commercially until 1860 and then not in an appreciable amount until the first years of the present century. Extensive planting followed the war.

Douglas fir is also one of the most valuable species in the United States. At present it comprises about one-fourth of our remaining merchantable timber, and its continued existence, owing to its ease of reproduction, its rapidity of growth and its simplicity of management, is of paramount importance to the future softwood supply of this country and to the economic well-being of the Pacific Northwest.

BRITISH NAMES FOR AMERICAN CONIFERS

In the United States only one species of Douglas fir, *Pseudotsuga taxifolia* (Lam.) Britt., is recognized throughout the West. In Great Britain and in fact over all of Europe this American species is considered to include two distinct species and one variety, that is, *P. douglasii* Carr., commonly called coast, Pacific Coast, Oregon, or green Douglas fir; *P. glauca* Mayr. known as mountain, Rocky Mountain, Colorado, or blue Douglas fir; and *P. douglasii* Carr. var. *caesia* Schwer., termed Fraser River or intermountain Douglas fir. The name Fraser River is an unfortunate misnomer, since the Fraser River has its source in the northern Rocky Mountains and empties in the Pacific Ocean, so that green Douglas fir as well as the intermountain variety are found along its banks, and probably the blue Douglas fir besides.

It is not within the province of this paper to discuss the relative merits of the American and European species-concepts of Douglas fir. The latter has been well presented by Henry and Flood (8) and Hickel (9). Suffice it to say that the two species as recognized in Europe are at least biologically so different, particularly in their rate of growth and reaction to disease, that in this paper it seems advisable to adopt the European classification for the sake of clarity.

The British also subdivide certain other species of American conifers, and in other cases the specific name used is not in accordance with the one accepted here. A table of synonyms for a few of the more important species on which there is a difference of opinion follows:

AMERICAN NAME	BRITISH NAME
<i>Abies concolor</i>	<i>Abies concolor</i>
White fir	Colorado white fir
<i>A. concolor lowiana</i>	<i>A. lowiana</i>
Pacific white fir	Low's white fir
<i>A. lasiocarpa</i>	<i>A. sub-alpina</i>
Alpine fir	Alpine fir
<i>Pinus contorta</i>	<i>Pinus contorta</i>
Lodgepole pine	Beach pine
	<i>P. murrayana</i>
	Lodgepole pine

<i>P. radiata</i>	<i>P. insignis</i>
Monterey pine	Insignis pine
<i>Tsuga heterophylla</i>	<i>Tsuga albertiana</i>
Western hemlock	Hemlock spruce

IMPORTANT DISEASES

Canker

A most important disease primarily of Douglas fir is canker, presumably caused by *Phomopsis pseudotsugae* Wilson. The effect of this fungus on the host, resulting in die-back of the young shoots and canker of the stems, has recently been adequately described and figured by Wilson (27). The parasite has probably been present in Europe under various names for many years but it is only recently that it has received serious consideration, when with the extensive planting of Douglas fir in Great Britain the damage caused by the fungus has become appreciable. Rostrup (19), in 1890, described *Phoma pithya* Sacc., which he considered identical with *Phoma abietina* Hart., attacking the shoots of Douglas fir and silver fir in Denmark, and in 1906 (20) listed it from Bornholm, a small island in the Baltic Sea about 100 miles east of Copenhagen. However, specimens collected on this island by the writer in September, 1925, from Douglas fir, said by the head forester to be infected with *Phoma pithya*, were found upon critical study to be attacked by *Phomopsis pseudotsugae*. Böhm (1), in 1896, described and figured a canker disease on Douglas fir in Germany which he attributed to *Phoma abietina*, but which from his description is the same as *Phomopsis pseudotsugae* in its effect on the host. Somerville (22) mentions *Phoma pithya* on Douglas fir in Scotland as early as 1898. Hickel (9, p. 31), mentioning the diseases of Douglas fir in Europe, lists *Phoma pithya*, *P. abietina*, and *Phomopsis pseudotsugae* as synonymous; while Visart and Bommer (24, p. 302) report *Phoma pithya* on Douglas fir in Belgium. De Koning (13), in 1922, records *Phoma pithya* on Douglas fir in Holland. In France, Prillieux and Delacroix (18), in 1890, described *Fusicoccum abietinum* (Hart.) P. and D. on silver fir which later was shown to be identical with *Phoma abietina*.

Wilson (27, pp. 13, 28-31), in his bulletin on *Phomopsis pseudotsugae*, recorded the fungus on Douglas fir and Japanese larch, cited a single instance on European larch, and reported one of *Diaporthe pithya* Sacc., presumably the ascigerous stage of *P. pseudotsugae*, attacking lowland white fir (*Abies grandis*). Wilson separates the species on the following basis.

Phoma abietina (from specimen determined by Hartig). With sporophores, conidia $12-14 \times 5-6 \mu$, somewhat angular in outline.

Phomopsis pseudotsugae (from specimens). With sporophores, conidia $5.5-8.5 \times 2.5 \mu$, regular in outline.

Phoma pithya (from description only). No sporophores.

There is also a difference in the structure of the pyrenidium of *Phomopsis pseudotsugae* and *Phoma pithya*. A critical study of the type collection of *P. pithya* may show it to be identical with *Phomopsis pseudotsugae*.

The three species are so closely related that they have been badly confused. It will require intensive work to straighten out the present confusion in nomenclature in order to determine the history and distribution of *Phomopsis pseudotsugae* in Europe. The fungus is actually present in Great Britain and Denmark, and for practical purposes it must be considered as present in Germany, Holland, Belgium, and France. In Great Britain the canker was found to be much more common in the comparatively moist, cool regions of Wales and Scotland than it was in the dry, warm parts of England such as Norfolk, Suffolk, and Hampshire. This indicates that the disease will be found more widespread in northern than in southern Europe. As yet the fungus is not known in North America, although Hahn (6) has recently found another *Phomopsis* on Douglas fir in nurseries in the eastern United States. This investigator is now in Great Britain studying the problem further.

So far, the canker is essentially a disease of young trees, and no indications of it have been found on large, mature individuals. Furthermore, it is practically confined to the green form and rarely attacks blue Douglas fir. This was strikingly apparent in the infection on Bornholm. Quite severe damage has occurred in plantations, just after the trees have been set out, by the killing back of the leading shoot, resulting either in the death of the tree or a bunchy-topped, slow-growing survivor. Larger trees may be killed by the canker girdling the stem, but no tree over 2 inches diameter breast-high was seen killed in this way. As a rule most trees which have succumbed were less than 8 feet in height. The largest tree seen with a stem canker was 8 inches diameter breast-high just above the canker. This was in Denmark.

There are two ways in which stem cankers originate: through the tip infection of a living lateral with subsequent spread to the stem, and by direct inoculation of the stem through a wound. There is no evidence that infection can occur through uninjured bark. While the former method of infection on the stem of seedlings and small saplings is not uncommon, it is not frequent in stands varying from the large sapling to small pole size where very few living lateral shoots are to be found on the lower stems, the branches having been uniformly killed by shading. In such stands wounding is all-important: without wounds the trees remain practically

free from canker. Wounding, nearly always the result of human carelessness, can be prevented and the canker on stems of trees from large sapling to small pole sizes largely controlled. The two worst cases of stem cankers observed were in Wales, where a stand of large saplings had been badly wounded by pruning, and on Bornholm, Denmark, where many wounds had occurred from thinning. In both plantations practically every wound had resulted in a canker.

The Japanese larch was occasionally found attacked by *Phomopsis pseudotsugae*, but always in plantations that had been pruned or thinned. Only one form of the disease was seen, that is, cankers on the main stem. No killed trees were noted. No die-back of the tops or branches was observed. The cankers on Japanese larch are not quite like those on Douglas fir. The most striking difference was that of greater size, the cankers on larch sometimes having a vertical extent of 2 feet, while those on Douglas fir rarely exceeded 8 inches. Furthermore, the margins of the cankers on larch were marked by a pronounced exudation of resin.

This parasite would be an extremely dangerous one, capable of killing trees up to a considerable size except for the fact that canker development persists only through the winter and spring following infection and apparently stops completely when the host resumes active growth in the spring. It is noteworthy that the fungus *Neofabrea malicorticis* (Cordley) Jackson, causing a similar canker on apple trees in the Pacific Northwest, behaves in the same way (12, pp. 178-179). Wilson (27, p. 21) reports a single exception to the foregoing habit—the case of a canker which continued development over a four-year period. Since the cankers develop rather slowly, particularly horizontally, it is possible for only a rather small stem to be girdled, unless several cankers coalesce, which does not often happen.

The most serious damage seen by the writer was near Murthly, Scotland, where a plantation of 10- to 11-year-old Douglas firs was only about 50 per cent stocked, on account of killing by *Phomopsis pseudotsugae* during the years 1920-1922. The bare spots had been replanted in 1921, but most of the new trees had been immediately killed. This is a very serious loss, judged even by present American standards which are necessarily less rigorous than European ones.

Our standard is a fully stocked stand at maturity, so that any disease which does not reduce the final stocking or the increment can not now be considered of importance, since thinning is not economically practicable. But in Great Britain, where it is hoped to realize on thinnings, the death of any young trees in a plantation is a loss, particularly when planting costs due to drainage work, cutting ferns and brambles, and rabbit fencing, trapping or shooting amount to from 4 to 10 pounds (approximately 20 to 50 dollars) per acre, as contrasted with 15 dollars on national forests in the Pacific Northwest.

But in most of the infected areas seen throughout Great Britain the actual damage would be considered slight if occurring in stands of identical age and size in the Douglas fir region of the Pacific Coast. Nevertheless this parasite must be considered a serious potential danger to the Douglas fir in North America, for enough was seen in Great Britain of the virulence and change in life habits of imported parasites more or less innocuous in their native haunts to realize that in general it is hazardous to predict what any fungus will do in a new environment. To be sure, the artificially created (planted) stands of Europe are inherently more subject to disease than the naturally regenerated stands of western North America, but, on the other hand, the discontinuous nature of the stands abroad is in itself a measure of protection against parasitic attack, while the continuous area of practically pure Douglas fir extending over hundreds of square miles in this country is an ideal situation for an epidemic. Considering the fact that Douglas fir at present comprises about one-fourth of all the merchantable timber in the United States and will always occupy a highly important position, there is much at stake.

Consequently this disease must be studied further. It is not yet proved by inoculations that Douglas fir canker is actually caused by *Phomopsis pseudotsugae*, the evidence so far being constant association of the organism with the disease—excellent as far as it goes. However, information is not complete on the method of infection, origin of the disease, its original host, its present distribution, and its relation to somewhat similar diseases which have figured in European literature. The origin of the fungus is extremely important. If it could be proved that it came from the Douglas fir region of North America, our problem would be solved: the parasite would no longer be a potential danger. In the writer's experience in the Douglas fir region, nothing similar has been seen, and it is his reluctant opinion that the organism is a European or other foreign fungus, normally saprophytic, which has become parasitic on two introduced species, Douglas fir and Japanese larch.

Needle Cast of Douglas Fir

Needle cast caused by *Rhabdocline pseudotsugae* Syd., first described by Weir (26) and later named by Sydow (23, pp. 194–195), has so far been reported in Europe at only two places—near Stobo, Peeblesshire, Scotland, and at a point 8 miles distant to which it has apparently spread from Stobo (29). The fungus was probably brought in directly from western North America where it is ubiquitous, since importations of Douglas fir have been made in the past to the place where the parasite was found.

The disease seemed even more virulent than in America, a heavy attack of it for four successive years having reduced the height growth of the most

severely attacked trees to a few inches per season. The intermountain Douglas fir was most severely injured, followed by the blue variety, while the valuable green form was not affected. If the parasite continues in this way, neglecting green Douglas fir, it will not cause much damage in Great Britain, since planting of the other two varieties of Douglas fir will be very restricted owing to their slow growth. There is of course a continual danger that a strain of the fungus to which the green Douglas fir is susceptible may be imported or that the present strain may ultimately adapt itself to the green form.

Douglas Fir Aphid

The most serious injury to green Douglas fir observed was caused by the aphid (*Chermes cooleyi* Gill.). This insect, originally imported from North America where it occurs throughout the range of Douglas fir, was found in greater or less degree in practically every Douglas fir plantation visited in Great Britain. It was not noticed on the island of Bornholm in Denmark. It has been described by Chrystal and Storey (3).

The insect rarely, if ever, kills the trees attacked, but where infestation is heavy there is a decided suppressing effect on individuals and stands. No trees escape in a heavy infestation. One plantation of trees from 10 to 15 feet high which normally grew from 15 to 36 inches per annum has been reduced for the past two years to an average annual height growth of from 3 to 6 inches. In another plantation of Douglas fir mixed with western red cedar the first named species was badly overtopped by the latter, due to *Chermes cooleyi* on the Douglas fir, while under normal conditions the Douglas fir would have grown much faster than the cedar. Infestation was always most severe in open stands or on the marginal trees in dense stands.

This insect in Great Britain is another classic example of a marked change in life habits of a parasite in a new environment. In North America it is practically innocuous, while in Great Britain it has become extremely virulent on the green Douglas fir only, rarely being found on the blue form. Furthermore, in America it also attacks Sitka spruce, causing cone-like galls on the twigs and at times severely injuring young trees, but in Britain the Sitka spruce strain has not yet been found, although young Sitka spruce is growing in many places immediately adjacent to or in the immediate vicinity of infested Douglas fir. Of course there is still some doubt as to whether the form on Douglas fir and the gall-causing form on spruce are actually one species.

Just what the future of this parasite in Great Britain will be is problematical, but from present indications, when the epidemic gains momentum, the consequences will be serious. Even though older plantations may be little affected by the attack, there will be a marked reduction extending over

several years or more in the annual increment of younger plantations, necessitating a corresponding increase in the rotation. Hope of control lies in the introduction from North America of the natural parasites of the insect and the selection of favorable sites for Douglas fir plantations.

Silver Fir Aphid

The silver fir aphid (*Dreyfusia nuesslini* Bödner) was apparently introduced into Great Britain from Continental Europe where it is widely distributed but not exceedingly virulent on silver fir. In 1925 the aphid was widespread in Britain, the damage to silver fir being so severe that the further planting of this species may have to be discontinued.

Needle and Twig Blight of Firs

Rehmiellopsis bohémica Bub. and Kab., recently discussed by Wilson and MacDonald (28), attacks the needles of the current year on several of the true firs. In severe infections the majority of the season's needles on each shoot are killed, resulting in the death of the shoot during the course of the growing season. If a severe attack is sustained over several seasons the stand is badly injured and many trees may die.

This was the case with a young stand of silver fir at Inverliever, Scotland. Here the fungus, working in combination with the silver fir aphid, was so steadily killing a stand of young trees up to 20 feet high that in a few years the entire stand would be wiped out. These trees, however, were on a very unfavorable site, which in the writer's judgment had much to do with the sustained intensity of the attack. On Bornholm, where silver fir is grown extensively on good sites, there had been an epidemic of this fungus during the 1924 season, but for some years previous it had been little in evidence, and an examination in September, 1925, showed that the summer's attack had been very light.

The only American species found attacked by *Rehmiellopsis bohémica* was noble fir. A small plantation on a poor site in Scotland was being severely injured in spite of attempts at control by cutting out the diseased portions. The aphid was not present on these trees.

Needle and Twig Blight of Pines

At various places in Scotland certain pines were suffering rather severely from needle and twig blight of pines caused by *Brunchorstia destruens* Eriks., which appeared to be spreading. The fungus was described on the continent years ago by Brunchorst (2) and Schwarz (21), the latter considering it to be the imperfect form of *Cenangium abietis* (Pers.) Duby, but it has only recently come under Dr. Wilson's observation in Great Britain.

At one place in Scotland where the parasite was observed, Swiss stone pine (*Pinus cembra* L.) was being so badly damaged that it seemed only a question of time until this species would be eliminated from the locality. The fungus was killing the tops and branch tips of the trees from 5 to 20 feet high to such an extent (the oldest about 25 years old) that many were dying and all were in more or less unhealthy condition. Swiss mountain pine (*P. montana* Mill.), western white pine (*P. monticola* Dougl.) and lodgepole pine were attacked, although not so severely as stone pine. The trees were all occupying a very poor site.

On another estate Corsican pine was being severely injured, while the mountain pine was less seriously affected.

White Pine Blister Rust

Very little white pine blister rust (*Cronartium ribicola* Fisch.) on 5-needle pines was seen in Great Britain, for there were relatively few pines of this group to be found. The disease was abundant in places on cultivated black currant (*Ribes nigrum* L.). Planting of 5-needle pines has been definitely abandoned because of blister rust, the opinion of British foresters being that it was poor policy to plant any species that would have an added charge against it for protection, when other species could be substituted. In the writer's opinion, there are places in Great Britain where 5-needle pines can be grown with little or no increased cost because, on the whole, wild currants or gooseberries were found to be rare.

On the island of Bornholm in Denmark, eastern white pine (*Pinus strobus* L.) was badly damaged by blister rust. No wild currants or gooseberries were seen; infection came entirely from European black currants in farm gardens at distances of 300 to 3,300 feet from the pines, no difference in degree of infection of the white pines being apparent as the distance from the currants increased. This locality afforded the most valuable evidence of the terrific damaging power of the European black currant. This currant can not be tolerated in a region where white pines are grown.

Needle Cast of Larch

Needle cast, very common on young European larch throughout Great Britain, is caused by the fungus *Meria laricis* Vuill. It results in the death of needles on young trees and particularly of the young needles on the long shoots. It was most serious in nurseries, where heavy losses have occurred during the past few years, seedlings and transplants being killed by defoliation. The fungus in Great Britain has been described by Hiley (11).

Larch Canker

In the past, canker on European larch caused by *Dasyscypha calycina* (Schum.) Frick. had been so destructive in Great Britain that the future

cultural value of this tree seemed very uncertain. In fact considerable damage is still being done. This disease has been described by Hiley (10, pp. 16-79).

But foresters, in the light of long experience, now feel that in the main this disease can be controlled by proper silvicultural methods. Larch has been planted indiscriminately on all types of site; it has been planted too thickly and stands have been allowed to stagnate. By avoidance of low, poorly drained sites, by wider spacing and judicious mixture in planting, and by early thinning in order to admit plenty of light and air, it is believed that relatively healthy stands of European larch can now be grown.

Cedar Leaf-Blight

Leaf-blight of western red cedar caused by *Keithia thujina*, which has been discussed by Weir (25), has been found during the past few years at a number of widely isolated points in England, Scotland, and Ireland (4, 5, 14, 16, and 17). As yet little is known as to what it may do, but so far wherever it has occurred it has been extremely severe. The parasite was apparently introduced from America, but the British authorities are somewhat puzzled by its sudden appearance in remote localities where no nursery stock has been known to have been introduced.

Root Rots

The two important root rots were caused by the honey fungus (*Armillaria mellea* (Vahl.) Quel.) and by *Fomes annosus* (Fr.) Cke.

The honey fungus seemed to attack all species and sizes of conifers although young trees in the sapling stage were most susceptible. It was particularly bad in plantations of conifers on cut-over areas previously occupied by old, overmature stands of oak. Much oak forest in Britain is being converted into softwoods. Corsican pine was by far the most susceptible of all the conifers observed.

Fomes annosus was also found attacking various species of conifers, being particularly severe on Norway spruce, silver fir, and Douglas fir. While trees up to 6 inches diameter breast-high and larger were seen which had been killed by the fungus, those in the sapling stage suffered most. Japanese larch was not immune from this fungus.

Chestnut Blight

The discovery of *Endothia parasitica* (Murr.) A. and A. on chestnut in Belgium and on a staging pole in London by Metcalf (15) in 1923 indicated that the fungus might be present in Great Britain, but, although chestnut was examined at various places, the disease was not found. The chestnut orchards in Kent were not visited.

The foregoing discussion on a few important forest tree diseases is merely to call attention to some parasites in Great Britain which are potentially dangerous to this country and to present examples of how introduced parasites can change their habits, particularly their virulence, in a new environment.

STRAINS OF FOREST PARASITES

Even a superficial study of forest tree diseases in Europe, particularly those introduced from North America, clearly points to the existence and importance of strains, biological species, or physiological species in forest tree parasites. Among the European organisms Hiley (10, p. 79) mentions two different growth forms of larch canker (*Dasysecypha calycina*), a saprophytic and a parasitic form.

Throughout the native range of Douglas fir the needle-cast fungus is found. In Scotland the fungus was attacking only the blue and intermountain varieties, neglecting the green. The Douglas fir aphid, on the other hand, so far has virtually confined itself to green Douglas fir throughout Great Britain, very rarely being found on the blue form, while in its native home it infests all forms of the host. *Phomopsis pseudotsugae*, presumably a native to Europe, was not found attacking blue Douglas fir, although it occasionally does, but was common enough on green Douglas fir.

Fomes annosus was commonly killing young conifers in Great Britain and Denmark. This fungus is often found in the Pacific Northwest on old stumps or dead trees and occasionally causes root rot and butt rot in a mature tree, but it very rarely infects young trees.

Black poplar (*Populus trichocarpa* T. and G.) in the Pacific Northwest is commonly infected by the yellow leaf blister caused by *Taphrina aurea* (Pers.) Fr., which is also found on the Lombardy poplar (*P. nigra* L. var. *italica*) in the same region but frequently not in the same locality. The fungus was observed in a nursery in Scotland on the leaves of saplings of *P. generosa* Henry, *P. laurifolia* Led., and *P. nigra*, about 4 feet high. *P. nigra* was most heavily infected. *P. trichocarpa* in the same block and with its leaves intermingled with the diseased *P. generosa* was absolutely free from infection.

The foregoing conditions show clearly that strains do exist in certain species which are represented in America, on the polymorphism of which there is little or no information. It is, of course, much easier to see this in Great Britain, where the various growth forms of conifers of wide distribution are planted side by side, than it is in America, where the species occur naturally over thousands of square miles and direct comparison is rarely possible. These conditions also indicate a pathological as well as a

morphological basis for the separation of Douglas fir into two distinct species as is done by the British.

QUARANTINE PROTECTION

In the past, forest trees, particularly conifers, have been imported indiscriminately into Great Britain from all over the world, and in some measure this is still going on. Neither the quarantine laws nor the inspection force are adequate, while there are the usual difficulties in properly enforcing such laws as do exist.

Fortunately, or unfortunately, Great Britain has never had a disastrous plant disease epidemic. If she had, the country might be awake to the need for more careful regulation of the importation of nursery stock. The elm disease, probably caused by bacteria (*Micrococcus ulmi*) and probably introduced during the World War, is now rapidly killing the trees in Holland and nearby sections of Germany, Belgium, and France, but has not yet crossed the channel.

But some very dangerous diseases have been brought in. From North America have come the Douglas fir aphid and the Douglas fir needle cast, the former already wide-spread and serious on, and the latter potentially dangerous to, Douglas fir, while the cedar leaf-blight is becoming increasingly bad on western red cedar. From continental Europe the silver fir aphid has arrived.

It would seem that the discontinuous nature of the stands would greatly retard the spread of those diseases practically confined to one host species. This would probably be so except for the indiscriminate movement of nursery stock about Great Britain and Ireland, in some cases even when it is known to be diseased. Great Britain, occupying an isolated island, has had a good opportunity to keep her forests free from foreign parasites. This chance has been largely lost by the unregulated importation of young trees.

There is a lesson for the United States in this. Our quarantines are the only safeguard that will prevent the introduction of several known, potentially dangerous forest tree diseases and undoubtedly many of which we have no knowledge. Any weakening of our quarantine laws or policy will result in increased future expenditure for control. Another point of primary importance must be considered. It may be just as dangerous to introduce a foreign strain of an indigenous parasite as it would be to let a new species come in. For example, the strain of *Fomes annosus* observed in Great Britain, if introduced into the Douglas fir region of the Pacific Northwest, might prove highly virulent, whereas the fungus as it exists there at present is of trivial importance.

FACTORS FAVORING DISEASE

In silviculture in Europe, and particularly in Great Britain, certain practices are common which predispose stands to diseases of epidemic character. It is axiomatic in crop production that intensive culture increases the danger from disease, and timber growing in western Europe is decidedly intensive.

Clear cutting followed by planting, almost universal in Great Britain, in itself predisposes stands to attack by fungus and insect parasites. In Denmark natural regeneration is practiced wherever possible, and it was noteworthy that Skovrider K. Bramsen, in charge of the state forest of Almindingen, repeatedly emphasized the greater amount of disease, particularly root rot caused by *Fomes annosus*, in the planted stands as compared to those established by natural regeneration. In Switzerland clear cutting is prohibited by law. The complete and sudden exposure of the forest soil resulting from clear cutting is considered very detrimental, resulting in a continuous lowering of the thrift of the timber crop over successive rotations.

In addition, on private holdings the British are tending toward a short rotation, as low as 40 years where possible. Furthermore, species are planted pure to a large extent, although foresters are striving more and more to find suitable mixtures. Pure stands are the most susceptible to fungus and insect attacks, consequently much of the woodland in Great Britain, consisting of pure planted stands managed on a short rotation and clear cut with the consequent exposure of the forest soil at more frequent intervals, is inherently susceptible to disease. The present silvicultural and economic ills of the pure, planted Norway spruce forests of Saxony are a classic example of the price that may finally have to be paid for continuous clear cutting and planting of a single species on a short rotation.

Proper selection of site is also very important. Since most of the conifers grown in Europe have been introduced from other regions, it was not known to just which sites the various species would be adapted, and at first little attention was paid to this. The results in many cases were disastrous. Stands on unsuitable sites grew slowly, were not thrifty, and sooner or later largely succumbed to insect or fungus attack. This has been well illustrated by Scotch pine, European larch, and silver fir.

Larch planted in dense, pure stands on low, moist or otherwise unfavorable sites with poor soil suffered severely from larch canker, while stands on favorable sites with good soil remained practically free from disease. To be sure it is not always possible to determine immediately the most suitable site for an introduced species, but sometimes even reasonable care has not been taken. A young stand of silver fir was seen in Scotland planted

on an exposed site in very poorly drained peat soil. The trees were rapidly being destroyed by two needle and shoot parasites, *Rehmiellopsis bohémica* and *Dreyfusia nuesslini*. Again in Wales, Douglas fir was found planted in soil only about a foot deep with an underlying, impervious, clay subsoil. The trees about 10 to 15 feet high were so far thrifty and growing rapidly, but it is a reasonable prediction that before many years there will be heavy losses in this stand from uprooting by wind or wet snow, if nothing else. Already damage by the latter has begun. In addition, the stand will ultimately stagnate.

The source of seed is another factor which has been too little considered in the past, but British foresters now feel that the inferiority of some of the present stands of Scotch pine and European larch is due to the fact that the seed came from localities on the Continent well outside the range for the optimum development of the species. Such inferior stands, of course, are quite susceptible to disease—for example, the susceptibility of the larch to canker. This general principle must be recognized and carefully considered for all species.

So far in America it has not been necessary in most forest regions to consider seriously the questions discussed above, but as silviculture here becomes more intensive and European practice is approached, the same difficulties will arise in some cases. However, as long as the majority of our managed forests of the future in this country are composed of native species naturally regenerated, with as little deviation from the original composition of the stand as possible, losses here from indigenous parasites should not be excessive.

CONCLUSION

There is one great lesson for American forestry in the existing situation in Great Britain: protect our native species.

The introduction of foreign nursery stock is a most dangerous practice; it may at any time result in another disastrous epidemic similar to chestnut blight or white pine blister rust. Foreign species should be introduced only as seed and the stock grown here. But any exotic tree is an uncertain quantity and if introduced should be grown only experimentally for a long term of years, for it is not until the end of the rotation that final judgment can be rendered as to the success or failure of an introduction. First, there is the problem of securing seed of the best quality from the optimum, native range of the species; second, the difficulty of proper site selection; and, finally, the possibility that the species sometime in its life may be attacked by an indigenous parasite hitherto innocuous. Any one of these factors can spell failure. For the native species, seed collection plays a minor part since natural regeneration is depended on mostly, the optimum sites are

known, and the indigenous parasites, while quite severe in some cases, never threaten the existence of any species. Those who suggest introducing a foreign species to replace a native tree already attacked by disease, instead of protecting the latter, if that is possible, are in a large measure advocating deferred trouble.

The introduction of an exotic tree species is, on the whole, a hazardous undertaking, but it is far more so in this country than in Europe, owing to our extremely inadequate knowledge of the suitability of foreign species to the various forest regions of the United States. Abroad, different exotic tree species had been grown in arboreta for a long time before they were established as components of the forest. This was particularly true in Great Britain, and is a practice which should be generally initiated at once by our own country where there are few arboreta, especially in the western states. Costing little to start or maintain, an arboretum nevertheless requires a long time to develop, since raising trees from seed is a slow process, and complete results can not be expected until the trees are mature. Arboreta or experimental plantings for testing exotic species should be maintained by all the forest schools and forest experiment stations. No other activity will yield a greater commensurate reward for the expenditure involved. In the future, circumstances which can not be foreseen or controlled may compel the widespread establishment of a foreign tree species. Only with knowledge gained from arboreta and experimental plantings can such a situation be adequately met.

Finally, by carefully following literature, corresponding with foreign workers, and actually studying conditions on the ground in those countries most closely united commercially with our own, forest pathologists in this country must obtain an extensive knowledge of exotic diseases of trees. In this way, if a foreign parasite is introduced, and we already have a knowledge of its life history and habits, priceless time can be saved in its detection and the initiation of eradication or control measures. The value of such information at the outset of the white pine blister rust or the chestnut blight epidemics, for example, would have been beyond estimation.

SUMMARY

1. This paper presents observations during 1925 on the pathological condition of various conifers introduced into Great Britain and Denmark, particularly species from the western United States.

2. Douglas fir is attacked by *Phomopsis pseudotsugae*, *Rhabdocline pseudotsugae*, and *Chermes cooleyi*; silver fir suffers severely from *Dreyfusia nuesslini*; various true firs are injured by *Rehmiellopsis bohémica*; while pines are damaged by *Brunchorstia destruens*.

3. In Great Britain planting of 5-needle pines has been discontinued on account of the ravages of *Cronartium ribicola*; and on the island of Bornholm, in Denmark, eastern white pine was found severely infected by this rust. Nursery stock of European larch is commonly defoliated partially or completely by *Meria laricis*, while the older trees are frequently cankered by *Dasyscypha calycina*. Western red cedar is attacked by *Keithia thujina*, causing leaf blight. Root rots caused by *Armillaria mellea* and *Fomes annosus* are common on conifers.

4. The existence of strains of certain forest tree parasites not regarded as polymorphic in North America is quite apparent in Great Britain.

5. Several dangerous forest tree diseases have been brought into Great Britain, which indicates the need for careful regulation of the importation of nursery stock.

6. Certain practices, such as the culture of pure stands of one species on a short rotation followed by clear cutting, predispose stands to disease. In addition, plantations have sometimes been established on poor sites. The source of seed also has not always received proper consideration.

7. Foreign species should be introduced into the United States only as seed and the stock grown here. But even this is hazardous, since the species may become subject to the attack of an indigenous parasite; consequently there is need for the establishment of extensive arboreta throughout the forest regions of this country where the behavior of exotic species can be studied.

8. Finally, forest pathologists in this country must obtain an extensive knowledge of exotic parasites on trees in order to safeguard our forests.

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A WITCHES' BROOM OF INTRODUCED JAPANESE CHERRY TREES

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INTRODUCTION

About 60 years ago the first Japanese cherry trees were imported into the United States, but few of them became established here at that time because the principal method of propagation which was tried involved grafting and budding them upon *Prunus avium* Linn. and *P. cerasus* Linn. which are not scitable grafting stock for Japanese cherries (24). Subsequently small numbers of them were obtained direct from Japan or through European nurseries (17). In 1906 (17) 25 varieties were imported by Dr. David Fairchild, who planted them at his home near Chevy Chase, Maryland. In 1912 shipments of 12 varieties of Japanese cherry trees were presented by the city of Tokyo to Mrs. Taft for the city of Washington (17, 19). These varieties (Mazakura, Yoshino, Shirayuki, Ariake, Mikurumugaeshi, Gyoiko, Fukurokuju, Ichiyo, Kwanzan, Takinioi, Fugenzo, and Jonioi) are described by Russell (17). According to a letter from him they are distributed in three *Prunus* species (*P. lannesiana* Wilson, *P. serrulata* Lindl., and *P. yedoensis* Mat.), which are described by Wilson (24). Over 2,000 of these trees, about half of which belonged to the Yoshino variety, were planted in Potomac Park, Washington, D. C., where they have grown very vigorously. A few Japanese cherry trees are growing in other parks and in private grounds in the District of Columbia, and some have also been planted as street trees in Chevy Chase, Maryland. Collections of them in other parts of the country are listed by Russell (17), and some varieties are now being sold by several nurseries. Since Japanese cherry trees constitute a real contribution to American horticulture, a discussion is not amiss of a witches' broom disease which was introduced with them before the enactment of our present plant quarantine laws.

Observations on this witches' broom at Washington were begun in 1917, after attention was called to it by Dr. T. Tanaka. The causal fungus appears to be *Exoascus cerasi* (Fuckel) Sadebeck.²

¹ The writer wishes to acknowledge the assistance of Dr. Carl Hartley and Mr. G. G. Hahn in collecting the data for this article.

² Miss Anna E. Jenkins, of the Office of Pathological Collections, made this determination from preserved mounts of stained sections prepared by G. G. Hahn from specimens of the disease on *Prunus yedoensis* which he collected in Potomac Park, Washington, D. C., on April 17, 1917. She was unable to compare these sections with those of an authentic specimen of *Exoascus cerasi* (Fuckel) Sadebeck, as such was not available but she found that the characters of this fungus agreed with published descriptions of it.

SYMPTOMS OF THE DISEASE

Japanese cherry trees when infected throughout have peculiar bunchy crowns (Fig. 1). Infected twigs bear no flowers but produce leaves earlier than uninfected ones. These leaves are undersized, much crinkled, and slightly discolored. About a week after the uninfected portions of the



FIG. 1. A Japanese cherry tree which had been transformed entirely into a witches' broom by *Exoascus cerasi*. The trees in the background were normal. Photograph made in Potomac Park, Washington, D. C., in April, 1917.

trees begin to flower, the under surfaces of the crinkled parts of the infected leaves become covered with powdery, bloomlike, inconspicuous, fungous coatings—the spore-bearing layers. The spore-bearing parts of the leaves turn black rather promptly. Later in the season normal leaves are developed. Figure 1 shows a tree which has been transformed entirely into a witches' broom. The trees in the background are normal. Douglass (4), in a popular account of this disease, published additional pictures of the disease on the Japanese cherry. Other writers (8, 9, 11, 13) have described the witches' brooms which *Eroasus cerasi* causes on other species of *Prunus*.

THE DISEASE IN THE VICINITY OF WASHINGTON

On April 18, 1917, every Japanese cherry tree in Potomac Park was carefully examined for symptoms of the disease. Nineteen scattered trees, all belonging to the Yoshino variety (*Prunus yedoensis*), were infected.

This means that only a small percentage of this variety, and a still smaller percentage of the total number, were infected. Seventeen of the trees were infected throughout, the infection apparently having occurred before the trees were shipped from Japan, and the other two trees had been infected for a long time. A minute examination of the trees near the diseased ones revealed no evidence of infection. Since the trees are all grafted ones (19) it is entirely possible that the disease had been introduced into them by grafting.

Sixteen of the infected trees were immediately cut down, their roots dug up and the whole burned. Some of them appeared to be releasing spores at that time. Two others were heavily pruned, planted in tubs and grown for a time in the pathological greenhouses for observation. All spore-bearing leaves soon died, leaving only apparently healthy ones.

The nineteenth tree had apparently been infected throughout when shipped from Japan. A secondary main shoot had arisen later from the main axis. This shoot was growing vigorously and appeared to be entirely healthy. The diseased part of the tree was removed and burned and the remainder left standing.

Subsequent inspection in 1918 showed a small witches' broom on a tree of the Yoshino variety which had apparently become infected in 1916, four years after its introduction into America. Only 1916 and 1917 growths were visibly infected. This broom was cut off six inches back of the first healthy twig and burned before any spores had been disseminated. No other evidences of infection were seen. All cherry trees in the park were again inspected in 1919 and 1924, and those of the Yoshino variety in 1925, but no further signs of witches' broom were found.

Some witches' brooms had been removed from the Japanese cherry trees on the Fairchild estate prior to 1917 and no evidence of recurrence was noted in 1917. At that time another small broom was removed from one tree of the green flowering variety known as Asagi (*Prunus lannesiana*). In 1926 these trees were inspected again but no evidence of this disease was noted.

The Japanese cherry trees which are growing as street trees in Chevy Chase, Maryland, have remained free from the disease. None of the ornamental cherry trees in the other parks in the District of Columbia which were inspected in 1917 were visibly infected by *Exoascus cerasi*.

Except for the two places previously mentioned, the disease has not been reported near Washington, D. C.

THE DISEASE IN GENERAL

The writer has been able to learn little in regard to the distribution and importance of the witches' broom which *Exoascus cerasi* causes in Japan, because most of the papers on the disease there are written in the Japanese language. It occurs on *Prunus pseudo-cerasus* var. *spontanea* Maxim, *P. pseudo-cerasus* var. *suboldii* Maxim, *P. miqueliana* Maxim and *P. cerasus* L. (14). Heinricher (8) and Laubert (11) consider the disease neither destructive nor important in Europe, while Tubeuf (22, 23) says that it has assumed considerable economic importance there because every cherry tree in a fruit garden may be attacked by it and each tree may bear several brooms. Its hosts have been listed by Hesler and Whetzel (9), Eneke (6) and others. The disease appears to be rare in England (3).

In the United States the presence of *Exoascus cerasi* was first reported in 1886 by Meehan (13) on an escaped cherry tree (*Prunus avium*) in Pennsylvania. Since then the disease has been reported from approximately half of the states but in most cases it has been described as occurring rarely, many of the reports being based upon the occurrence of one broom on one tree. However, according to Owens (15) and others (9, 10, 12), *E. cerasi* is very common in Washington and Oregon. The reported hosts for it seem to be *Prunus* sp. (1); *P. americana* (1, 16); *P. avium* (1, 13, 21); *P. cerasus* (1); *P. demissa* (1, 16); *P. emarginata* (18); *P. hortulana* (16); *P. pennsylvanica* (1, 16); *P. serotina* (1, 16); and *P. virginiana* (1). It is possible that some of the witches' brooms attributed to *E. cerasi* may have been caused by other fungi because other witches' brooms of cherry, such as those caused by *E. interstitiae* (2), also occur in this country and many of them were observed when they were not fruiting and apparently were not compared with authentic material of *E. cerasi*.

The facts that *Exoascus cerasi* was not reported in the United States until after the introduction of the first Japanese cherry trees and that the

first reported host was *Prunus avium*, one of the species which was used as grafting stock for them, are interesting although they may not be significant. *P. avium* is also one of the two most common hosts for *E. cerasi* in Europe (5). Whether the disease is native to this country is not known, but it is highly probable that it may originally have been introduced from either Europe or Japan.

There seems to have been little effort to eradicate systematically the witches' brooms from cherry trees in America (9) and few attempts even in the parts of Europe where the disease is considered of economic importance (22, 23). Duggar (5) recommends controlling the disease by spraying, while others (6, 9, 10, 12, 20) advise pruning and burning the diseased portions of the trees. Stevens and Hall (20) issue a warning against using portions of brooms in budding and grafting. These recommendations were made apparently as a result of general experience rather than as the result of specific experiments with *Exoascus cerasi*.

The attempts reported here to eradicate *Exoascus cerasi* from Japanese cherry trees seem to have been entirely successful despite the fact that in both cases the disease had been present and the pathogene probably sporulating for a number of years. The fact that *E. cerasi* was spreading slowly probably accounts for the immediate success of the eradication. Stewart (21) and others also have reported that the disease spreads very slowly in America, and Heinricher (8) and others that the same is true in Europe. It is very fortunate for cherry growers that, unlike *Endothia parasitica* (7) and some other introduced parasites, this introduced *Exoascus* has not found hosts or conditions which permitted more rapid spread.

SUMMARY

A witches' broom caused by *Exoascus cerasi* was introduced near Chevy Chase, Maryland, in 1906, and into the District of Columbia in 1912 on separate shipments of Japanese cherry trees. Most of the infections were on the Yoshino variety (*Prunus yedoensis*) but the Asagi variety (*P. lannesiana*) was also found infected.

Exoascus cerasi appears to spread very slowly in this locality. Probably because of this fact both of the above infections were easily and permanently eradicated by cutting out and burning the infected parts, although the disease in both cases had been present for several years before any attempt was made to eradicate it.

Reports of the occurrences of the disease in other localities are summarized.

OFFICE OF FOREST PATHOLOGY,

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NOTES ON HIBISCUS DISEASES IN WEST JAVA

CARL HARTLEY

Until recently, most species of the genus *Hibiscus* received little attention from pathologists. The indices of the first 41 volumes of the Experiment Station Record list under *Hibiscus* only three references to disease. The interest in Java jute (*Hibiscus cannabinus*) and roselle (*H. sabdariffa*) as fiber sources has recently resulted in attention to their diseases in Malaysia. The writer wishes to place on record some data on them obtained in the experimental plantings of the Departement van Landbouw incidentally to the investigation of some of the same diseases on peanuts for the Instituut voor Plantenziekten in 1921 and 1922.

The wilt or slime disease (*Bacterium solanacearum*) causes serious damage at Buitenzorg in an occasional field of *H. cannabinus*, but is never as serious as in peanut or tomato. The *Hibiscus* is very susceptible when young, but infected plants which live till they develop woody tissues in the stems make good recoveries. The relation of the bacteria found in *Hibiscus* to the strains of the same species which attack other hosts is discussed in a paper by Dr. M. B. Schwarz which is to appear as a Mededeeling of the Instituut voor Plantenziekten. This disease is known on *Hibiscus* in Deli (north Sumatra),¹ and what may be the same disease has been reported from the Malay Peninsula.²

Sclerotium rolfsii, or a near relative of it, is of rather frequent occurrence on *H. cannabinus* in the neighborhood of Buitenzorg. It was observed most frequently on plants whose early growth had been stunted by drought. Badly diseased plants appear to be weakened in general, have small leaves, and ultimately wilt; the abscission of lower leaves which occurs in plants attacked by *Bacterium solanacearum* is wanting. The lesions which are dry and show characteristic concentric red brown and pale gray brown zones, are sometimes found on the stems at considerable distances from the ground.

In a single instance there was observed a small local epidemic of another type of stem lesion on plants of *H. cannabinus* 25–30 cm. high. In contrast to the *Sclerotium* lesions these were gray and water-soaked, and immedi-

¹ JOCHEMS, S. C. J., and J. G. J. A. MAAS. Slijmziekte in de *Hibiscus cannabinus* op Sumatra's Oostkust. *Teysmannia* 33: 542–546. 1922.

² EATON, B. J. Pests and diseases of plants. Agr. Bul. Federated Malay States 9: 75–77. 1921.

ately produced in moist chamber a heavy coating of deep salmon pustules of hyalin spores ($13\frac{1}{2} \times 4\frac{1}{2} \mu$). This fungus was identified by Dr. A. Van Luijk as *Colletotrichum hibisci* Polacci.

Root knots were very abundant on *H. cannabinus* in parts of one planting. Nematodes in them were identified by Dr. R. Menzel as *Heterodera radiculicola* Greeff. Eel worms on this species have also been reported from Malaya.³

In parts of a planting of *H. cannabinus* on the coast near Terisi the plants were reported by Mr. De Veer almost universally affected with rosettes at the tips of stems which had previously made normal growth. The leaves were normal, but internode lengths were reduced almost to zero. Some of the plants examined by the writer had recovered, and were developing new tips and resuming normal growth. No causal agent could be found.

On a green stemmed race of *H. sabdariffa altissima* at Buitenzorg Dr. A. Horst called to the writer's attention a leaf disease resembling closely that recently described and figured by Palm and Jochems in Deli, Sumatra.⁴ The disease regularly began at the union of petiole and leaf-blade, and in Java tended to follow the veins. The death of all or a large part of the leaf blade usually resulted from a single lesion. Spread down the petiole was common, but not the killing of the main stem described on some of the plants from Deli. As appears to have been the case in Deli, most of the older leaves on the plants were destroyed by the disease, but too late in the season to hinder growth materially. The type of injury differed from that attributed to *Phoma sabdariffae*⁵ in that the latter seems to have been primarily a stem parasite. In both Java and Sumatra the diseased leaf tissue was darkened by the great number of pycnidia which developed. At Deli the pycnidia contained spores, but those examined by the writer in Java had failed to mature. Apparently pure cultures of the Buitenzorg fungus were obtained from the interior tissue at the end of a petiole lesion. These cultures also failed to develop spores on either agar or steamed rice, though on both substrata great numbers of the sterile pycnidia were produced. The fungus was examined by Dr. Van Luijk but was not identified. One of the cultures was used in 1921 in preliminary inoculations on the green *H. sabdariffa altissima*, on a red stemmed strain of it on which the disease had not been found, and on *H. cannabinus*. Inoculum from a culture on rice

³ EATON, B. J. *Loc. cit.*

⁴ PALM, B. T., and S. C. J. JOCHEMS. Een bladziekte van roselle (*Phyllosticta* spec. op *Hibiscus sabdariffa* L.) Indische Culturen 10: 391-393. 1925.

⁵ REINKING, A. O. Philippine economic plant diseases. Philippine Jour. Sci. (A) 13: 165-274. 1918.

was placed on the surfaces of the leaves and covered with wet cotton held in place by wire clips. Results were negative on *H. cannabinus* but positive on both varieties of *H. sabdariffa*. Positive results were secured most readily on leaves which had just unfolded. The lesions obtained produced the typical sterile pycnidia promptly. The experiments were controlled by parallel inoculations with a pink-spored fungus also isolated from dead *Hibiscus*. All of the control inoculations were negative.

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SWEET PEA FASCIATION, A FORM OF CROWNGALL

NELLIE A. BROWN

Fasciation of sweet pea plants has occurred in several successive years in some of the greenhouses of New Jersey. It has occurred in a greenhouse in New York State also, and a few plants of garden peas similarly affected have come to us from Maryland and Virginia. It generally appears during the rapidly growing period and before the heavy blossoming stage begins. Blossoming and full development are checked by it, and the plant becomes worthless to the florist or gardener. There is usually a main upright stem with normal leaves, on the crown of which a bunch of short, fleshy or wide stems is produced, giving the condition called fasciation. The fleshy stems give rise to misshapen and aborted leaves (Plate I, 1).

The thickened tissue was examined microscopically for fungi, bacteria, and nematodes, but none were found. Plates were poured from the thickened stems in the hope of isolating the crowngall organism. It proved to be present, and several colonies were picked off for inoculating. Typical conditions as they occurred in New Jersey were produced on sweet pea plants by one colony out of the four tried. (Plate I, 2 and 3.) The other three colonies were non-infectious. Inoculations with these three were made into 28 seedlings and 23 sweet pea plants.

The fasciation produced by inoculations was on the same variety of sweet pea, Zvolanek's rose, on which the disease occurred in New Jersey. The seed was purchased from an Eastern seedsman. The non-infectious colonies were tried on the same variety. The successful inoculations were made into seedlings which had been sprouted on moist blotting paper. Very fine punctures were made into the tender stems after which the seedlings were planted in pots. A very definite fasciation occurred on four out of eleven seedlings in from 40 to 60 days after inoculating. From these fasciations the organism was reisolated.

Inoculations made with the infectious colony into the crown of 14 sweet pea plants of the same variety when the plants were 5 to 8 inches tall did not produce a fasciated condition. Inoculations made into tobacco and geranium plants with the same colony were also negative. The organism seems to be in some respects a weak or highly specialized strain of *Bacterium tumefaciens*. It may be in the soil or may be carried on the

seed. If the disease has occurred once in a greenhouse, the soil should be disinfected or not used again for sweet peas.

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EXPLANATION OF PLATES

PLATE I

1. Sweet pea fasciation from New Jersey, enlarged about $1\frac{1}{2}$ times.

2 and 3. Fasciations produced on sweet pea plants by inoculating with the crown-gall organism isolated from 1. Inoculations made April 24, 1926. Photographed, July 2, 1926. Enlarged about 2 times.

PLATE II

Fasciation on a second crop of sweetpea plants in the same New Jersey greenhouse. In some parts of the house as many as ten per cent of the plants were affected. Nematodes were also present in the house but this disease is not due to them. $\times 2$ nearly.





Fasciation on a second crop of sweet pea plants in the same New Jersey greenhouse. In some parts of the house as many as ten per cent of the plants were affected. Nematodes were also present in the house but this disease is not due to them. $\times 2$ nearly.

EFFECTS OF SOIL MOISTURE AND TEMPERATURE AND OF DEHULLING ON THE INFECTION OF OATS BY LOOSE AND COVERED SMUTS¹

C. O. JOHNSTON²

In the experimental studies on smut of oats conducted at the Kansas Agricultural Experiment Station prior to 1924, it was constantly noted that smut infection never reached 100 per cent, no matter how heavily the seed was smutted. Infection in the hulled varieties seldom exceeded 50 per cent and in hull-less varieties it was seldom above 95 per cent. Other investigators have shown the marked dependence of smut infection in oats and barley on soil temperature and moisture and the absence of glumes. Some experiments were conducted at Manhattan, Kansas, in 1924 and 1925 to ascertain the importance of these factors on smut infection in oats under Kansas conditions.

MATERIAL AND METHODS

The seed used in these experiments was obtained from the Department of Agronomy, Kansas State Agricultural College. The varieties selected for the soil moisture and temperature studies, together with their usual reaction to smut as determined by experiments in preceding years, were as follows: Hull-less, Ks. 5201, very susceptible; Aurora, Ks. 5206, susceptible; Richland, Ks. 5209, moderately susceptible; Burt, Ks. 6004, moderately susceptible; Burt, Ks. 6090, resistant; and Kanota, Ks. 5179, resistant. All of these varieties, excepting Hull-less, are hulled oats and are of considerable economic importance. The same varieties, with the exception of the Hull-less, were used for the dehulling experiments. A selection of Kanota, with glumes only partially covering the caryopsis, was used in place of the Hull-less.

In the soil moisture and temperature experiment, half of the seed was inoculated with smut, the other half was clean. One row each of inoculated

¹ Contribution No. 240 from the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

² Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

The author is greatly indebted to L. E. Melchers for helpful suggestions rendered in the course of these experiments. These studies have been conducted in co-operation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

and uninoculated seed of each variety was sown on each date of sowing. In the dehulling experiment the glumes were removed from half of the seed of each variety, after which half of each of the hulled and dehulled samples of seed was inoculated. One row each of hulled-inoculated, hulled-uninoculated, dehulled-inoculated and dehulled-uninoculated seed of each variety was sown on each of two dates. Smutted and unsmutted samples of the same variety always were sown in adjacent rows, the unsmutted row thus serving as a check on the smutted row. All rows were 8 feet long.

The inoculum in both of these experiments was a mixture of about equal amounts of loose smut, *Ustilago avenae* (Pers.), and covered smut, *Ustilago levis* (Kell. and Sw.). The inoculum was prepared by grinding smutted panicles in a mortar and screening out all coarse chaff and seed. A quantity of the spores was added to the seed and the container vigorously shaken several minutes to give a uniform distribution of the spores. A very satisfactory covering of the seed with smut spores was thus obtained.

EFFECTS OF SOIL MOISTURE AND TEMPERATURE ON SMUT INFECTION

Jones (4) pointed out that spores of oat smut germinate best at optimum temperatures for the germination and growth of oats. Bartholomew and Jones (1) and Reed and Faris (5) found that a relatively low soil moisture content and high soil temperature were conducive to abundant smut infection. In order to determine the influence of soil temperature and moisture on the occurrence of smut infection at Manhattan, periodic sowings of oats were made throughout the season at intervals of about one week. The first sowings were made on March 1, 1924, and March 3, 1925, and the last on May 6, 1924, and May 4, 1925.

The soil temperature, taken at the time of sowing, was determined by means of a soil thermometer. This method is open to considerable error because sudden changes in temperature are not recorded and therefore the mean temperature for the germination period is not determined. The periodic readings obtained, however, are indicative of the general trend.

At each sowing, samples of soil were collected at the depth of sowing and their moisture contents determined. These data are given in terms of the percentage of moisture-holding capacity of the soil. The results secured in both years are given in table 1.

The spring of 1924 was late and wet at Manhattan, while that of 1925 was early and dry. These conditions are reflected in the low soil temperatures and high soil moistures recorded for 1924 and the high soil temperatures and lower soil moistures recorded in 1925. The percentages of smut in all oat-smut experiments were higher in 1925 than in the preceding year. In 1924 relatively low smut percentages were obtained in sowings made

TABLE 1.—*Effect of soil moisture and temperature on smut infection in oats, Manhattan, Kansas, 1924 and 1925*

Date of sowing	Soil temperature in degrees F.	Per cent smutted heads when seed was											
		Inoculated						Uninoculated					
		Hull-less Ks. 5201	Aurora Ks. 5206	Richland Ks. 5209	Burt Ks. 6004	Burt Ks. 6090	Kanota Ks. 5179	Hull-less Ks. 5201	Aurora Ks. 5206	Richland Ks. 5209	Burt Ks. 6004	Burt Ks. 6090	Kanota Ks. 5179
1924													
Mar. 1	38.9	1.9	2.0	0.4	0.0	0.3	0.8	1.4	6.0	0.0	0.0	0.3	0.0
10	36.9	0.0	4.6	0.0	1.0	0.0	0.2	0.0	1.9	0.0	0.4	0.3	0.2
15	33.2	16.3	3.9	0.0	0.0	0.0	0.3	0.7	2.0	0.0	0.4	0.0	0.0
24	38.0	63.8	17.6	2.7	7.8	3.1	2.0	2.3	9.8	1.8	8.7	0.3	0.3
Apr. 2	33.5	71.5	14.4	3.8	6.6	3.8	2.9	1.2	14.0	1.2	1.2	0.7	0.3
8	22.7	73.3	16.8	4.8	12.3	4.1	7.1	3.8	12.4	0.5	3.4	0.6	1.5
15	20.1	74.4	11.8	2.3	7.7	2.1	2.1	2.0	8.9	0.0	0.0	1.3	3.7
22	15.6	38.3	23.9	0.3	3.8	1.5	2.3	1.7	8.9	0.0	0.0	0.9	0.6
30	33.2	28.3	10.4	0.0	0.6	0.2	1.2	0.0	15.2	0.0	0.0	0.0	0.4
May 6	31.7	16.7	9.1	0.0	1.3	0.4	0.0	5.0	12.0	0.0	0.0	0.4	0.0
1925													
Mar. 3	41.5	27.1	14.9	4.1	15.0	1.6	0.3	0.5	3.4	1.1	1.2	2.4	0.0
11	43.5	64.7	21.3	7.0	21.2	2.3	0.0	5.1	8.5	3.9	3.3	0.7	0.0
16	40.5	92.4	34.6	12.7	43.7	5.5	0.6	3.6	9.8	14.6	10.5	0.8	0.4
23	56.0	94.8	37.0	20.9	47.3	2.7	0.6	16.2	12.2	14.6	10.3	1.2	0.3
30	50.0	87.7	29.1	16.5	32.1	6.2	1.0	6.9	20.9	14.5	5.7	2.8	0.5
Apr. 6	47.0	71.6	35.5	18.6	40.4	4.7	1.5	13.7	4.1	16.1	23.3	2.5	0.0
13	61.5	76.9	69.0	30.0	40.4	8.4	4.8	37.3	26.0	15.0	10.8	5.5	2.0
20	62.0	85.6	73.5	17.6	25.9	8.7	2.2	14.5	32.6	16.1	12.2	5.8	0.0
27	60.0	61.7	17.6	11.4	13.4	2.2	0.0	2.8	2.3	8.8	5.8	2.7	0.0
May 4	59.0	33.3	0.0	3.2	0.0	0.0	0.0	4.1	0.0	0.0	0.0	0.0	0.0

while the soil temperature was below 38 degrees F. The highest infections secured from inoculated seed that year were at temperatures of 62, 63 and 66° F. Highest infections were secured at soil moistures of 15.6, 20.1 and 22.7 per cent of the moisture-holding capacity of the soil.

In 1925 the highest percentages of infection were obtained at soil temperatures of 56, 61.5 and 62° F. It will be noted that the soil temperature was higher on March 3, 1925, than on March 24, 1924, and that all the early sowings in 1925 showed more smut than in 1924. The soil-moisture percentages did not fluctuate so widely in 1925 as in 1924. Sufficient data to determine the specific effect of varying soil moistures are not available, but the two years' results indicate that infection takes place most readily at low soil moistures.

The above discussion is based on the results secured from the use of inoculated seed. The fluctuations in the smut percentages where uninoculated seed was used are so large that a lack of uniformity of distribution of the smut spores on the seed is indicated.

EFFECT OF DEHULLING OF OATS ON SMUT INFECTION

Attention already has been directed to the difficulty of obtaining high smut percentages in hulled varieties of oats and the ease of securing high infection in hull-less varieties. Gaines (2), Gaines and Schafer (3), and Stanton, Stephens, and Gaines (6) have shown that much higher smut percentages can be obtained by removing the hulls from the oat kernels. Tisdale (7) and Tisdale and Tapke (8) proved that both loose- and covered-smut infection in barley could be increased by the removal of the glumes before inoculation. Experiments conducted at Manhattan, Kansas, in 1924 and 1925 confirmed these results for oats.

A quantity of seed of each of five varieties of oats was dehulled and half of it inoculated. Half of a hulled sample was also inoculated, the same amount of smut being used in each case. An inoculated and uninoculated row from both hulled and dehulled lots of seed were sown on each of two dates.

The varieties of oats chosen for this experiment consisted of one susceptible, two moderately susceptible, and three resistant varieties. One resistant variety was a loose-palea type of Kanota in which the caryopsis is only partly covered by the glumes, the tip being exposed. This is equivalent to the glumes being loosened, but not removed, from the seed. The data secured in this experiment are given in table 2.

In this experiment also the smut percentages were much higher in 1925 than in 1924. In every case but one there was a noticeable increase in smut where the seed was dehulled and inoculated. A slight decrease occurred in the dehulled Kanota in 1925. There was a large increase in smut in

TABLE 2.—*Effect of dehulling on smut infection of six varieties of oats. Manhattan, Kansas, 1924-1925*

Variety and number	Previous reaction to smut	Average per cent of smutted heads							
		Seed inoculated				Seed not inoculated			
		1924		1925		1924		1925	
		Hulled	De-hulled	Hulled	De-hulled	Hulled	De-hulled	Hulled	De-hulled
Aurora Ks. 5206	Susceptible	14.1	49.2	30.6	67.5	12.2	8.1	14.6	2.7
Richland Ks. 5209	Moderately susceptible	4.3	51.7	27.8	49.0	1.4	0.9	16.5	1.3
Burt Ks. 6004	Moderately susceptible	10.1	75.9	48.2	84.9	2.0	0.0	16.8	2.4
Burt Ks. 6090	Resistant	2.1	18.3	4.0	27.4	0.8	0.2	3.3	0.0
Kanota Ks. 5179	Resistant	1.9	6.6	2.1	1.8	2.4	0.9	1.8	0.0
Loose-palca Kanota Ks. 5179 sel.	Resistant	0.0	2.3	0.0	0.0

Aurora, Richland, the susceptible selection of Burt, and the resistant selection of Burt where the seed was dehulled and inoculated with smut before sowing. The increase was not so large in the resistant Burt as in the three susceptible varieties, but was much larger than that found in the two resistant strains of Kanota.

These data indicate that some varieties of oats, such as Burt Ks. 6090, which are usually considered as smut resistant, may merely be escaping infection through the protective qualities of their glumes. Others, such as Kanota, apparently have heritable protoplasmic resistance to smut, although their glumes also provide considerable protection. While dehulling the grains of susceptible varieties before inoculation increased the amount of infection, it still did not seem possible to procure 100 per cent infection, nor were the percentages obtained as high as those usually secured in hull-less varieties. It seems likely that hull-less varieties are heritably more susceptible than the hulled varieties used in these studies.

When the seed was not inoculated before sowing, there was a smaller amount of smut, in every case, in the rows from dehulled seed than in those from hulled seed. This probably is due to the fact that most of the spores are carried on or in the glumes and therefore are removed with them.

SUMMARY

1. Weekly sowings of six varieties of oats made during the seasons of 1924 and 1925 show that low smut infection takes place in very early and very late sowings.

2. High smut infection seems contingent on high temperature and low moisture content of the soil. Soil temperatures of 62° to 66° F. seemed to be most favorable for infection. Highest infections were obtained with the soil moisture below 30 per cent of the water-holding capacity of the soil.

3. Removal of the glumes from oats caused a large increase in the amount of smut infection. This increase was largest in susceptible and moderately susceptible varieties and much smaller in resistant varieties.

4. Comparison of the reaction of resistant Burt and Kanota when the glumes are removed indicates that the former derives part of its freedom from smut from the mechanical protection of its glumes, while the latter has a larger amount of heritable protoplasmic resistance. Thus it seems that some varieties of oats escape infection through the protection of their glumes, while others have protoplasmic resistance.

5. One hundred per cent infection was not obtained even with dehulled seed regardless of the heavy inoculation. This may be due to resistant individual plants in the hulled varieties used.

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BOOK REVIEW

Syokubutu-Byôrigaku, a Text-book on Plant Pathology is Romanized Japanese. By N. SUEMATU. Small octavo. 274 pp., 33 plates partly colored, and 153 figs. in text. Price \$2.60, postage incl. Published by Nippon-no-Rômazi-Sya, Hongô Komagome Akebonotyô 11, Tokyo, Japan.

The book is divided into a general and a special part. The former, which occupies almost two-thirds of the book, contains six chapters entitled: Plant Diseases in General, Bacteria, Fungi, Parasitism, Immunity, and Control of Diseases. The special part consists of six chapters which are concerned with the diseases of the rice-plant, wheat, barley, fruits, legumes, various other cultivated plants (like mulberry, tea, tobacco, etc.), bamboos, trees, ornamental flowers, etc.

The book is clearly and concisely written. It is richly illustrated. Some of the figures necessarily are copied from foreign sources; many of them, however, are original. The latter are photographs and drawings made from nature by the author himself and his friends—the results of their laborious work on the pathology of Japanese plants. Although the text itself, written in Japanese, may not be available to many American and European pathologists, these figures will be of much interest to them, especially as their explanation often contains the Latin names of the causal organisms.

—S. IKENO, Professor of Botany, Tokyo Imperial University, Japan.

**ABSTRACTS OF PAPERS PRESENTED AT THE EIGHTEENTH
ANNUAL MEETING OF THE AMERICAN PHYTOPATHO-
LOGICAL SOCIETY, PHILADELPHIA, PA., DE-
CEMBER 27, 1926, TO JANUARY 1, 1927**

Yellowing of alfalfa caused by leafhoppers. FRED R. JONES and A. A. GRANOVSKY.

In late June and early July, 1926, alfalfa "yellows" became unusually conspicuous locally in Wisconsin on the second crop of alfalfa. Several species of leafhoppers, especially *Empoasca fabae* (Harris) and *Deltocephalus inimicus* (Say), were abundant on the yellow plants. Since *Empoasca fabae* has been shown to initiate hopperburn of potato and apple, infestation experiments were performed to determine whether these insects were also responsible for alfalfa "yellows." Healthy alfalfa plants in cages in the greenhouse were infested with leafhoppers from the yellow alfalfa in the field. Insect-proof cages were constructed over several varieties of alfalfa in the field. The alfalfa in the cages was cut back, the cages were fumigated, and when new growth started half of the cages were infested with leafhoppers. Both in the greenhouse and in the field the infested alfalfa showed yellowing and dwarfing indistinguishable from that in surrounding fields; while in the uninfested cages alfalfa was vigorous and green. In autumn, the alfalfa crowns in the infested cages were conspicuously smaller than those in the uninfested cages, and many small seedlings of Grimm alfalfa were dead. These experiments have demonstrated beyond doubt that *Empoasca fabae* is responsible for alfalfa "yellows." The Wisconsin Experiment Station is continuing the study of this disease. (Cooperative investigations by the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Wisconsin Agricultural Experiment Station.)

Sugar beet curly-top virus, the cause of western yellow tomato blight. M. B. MCKAY and T. P. DYKSTRA.

The cause of western yellow tomato blight has remained a mystery for over a quarter of a century. Very striking circumstantial evidence in many fields during the past summer indicates that this disease is caused by the virus of sugar beet curly-top. Carsner reported tomato as susceptible to curly-top, but because only young plants were used and these died early no symptoms identical with western yellow blight were noted. Richards, of Utah, and others have observed the unusual correlation in the prevalence of sugar beet curly-top and western yellow blight of tomatoes in different years.

Recently the writers have induced healthy tomato plants to develop typical symptoms of western yellow blight under greenhouse conditions. Viruliferous leafhoppers, *Eutettix tenella*, from curly-top beets were placed on the healthy tomato plants. After an incubation period of two to three weeks, symptoms developed comparable to those so frequently observed in the field. These were general yellowing of the foliage, a rolling of the leaves, a purpling of the veins and a marked stunting of the plant. Some of the same leafhoppers which transmitted the disease to tomatoes also carried curly-top to sugar beets. The untreated controls have all remained healthy. (Cooperative investigation between the Oregon Agricultural Experiment Station and the Bureau of Plant Industry, Office of Vegetable and Forage Diseases.)

The control of plant disease through seed certification—aided by test gardens and field crop inspections. H. L. BOLLEY.

This paper is based upon the author's attempts to control certain diseases, particularly of potatoes, flax, and cereals, through breeding and selection under intensified conditions of disease infection. As early as 1896 special gardens for the accumulation and intensification of pathogens in soil and crop were prepared for the purpose of emphasizing "the survival of the fittest" through elimination of the non-resistant individuals. Through this method the author has introduced several strains of disease-resistant wheats and flax, capable of producing good yields under the most favorable conditions for infection. The work of other plant breeders also, including plant pathologists, agronomists, and practical plant selectionists, has progressed rapidly during late years, but the results on farms and in gardens are not what they should be, because it is impossible for the average farmer to keep the lines pure and disease-free.

The writer advocates protection of the work of plant breeding and crop improvement through seed certification, checked by the establishment of carefully planned gardens in which test plantings are made of diseased and disease-free pure lines for careful study preparatory either to greater distribution, or to certification, of those already widely distributed.

The effect of leaf rust, Puccinia triticina, on the seed production of wheat. E. B. MAINS.

Leaf rust often has been considered as causing little or no loss in production. By a comparison of a number of series of rusted plants with rust-free plants in the greenhouse from 1922 to 1925 it has been found that under some conditions there is a considerable reduction in the seed developed by rusted plants. The extent of the reduction depends on the severity of the infection, the infection period, and varietal susceptibility. When susceptible varieties are heavily infected from the seedling stage to maturity, little or no seed is produced. Severe infection from the beginning of heading to maturity has produced 15 to 25 per cent reduction in seed formation. It was found that the upper and lower spikelets in the heads of rusted plants usually failed to develop seed. The middle spikelets produced fewer seeds due to the failure of the development of the central flowers. Blossoming starts in the outer flowers of the spikelets and the middle spikelets of the head, progressing inwardly in the spikelet and up and down the head. While the first flowers to blossom in rusted plants are able to receive sufficient material for the development of seed, the later blossoms are starved and fail to develop seed. (Cooperative investigations by Purdue University Agricultural Experiment Station and Bureau of Plant Industry, U. S. Department of Agriculture.)

Susceptibility of wheat varieties and hybrids to wheat scab in Minnesota. J. J. CHRISTENSEN and E. C. STAKMAN.

During the past six years more than 250 varieties of *Triticum* spp., and a considerable number of selections from crosses, were subjected to an artificial epidemic of wheat scab at University Farm, St. Paul, Minnesota. The amount of seedling blight and head blight in the same variety in different years varied with the date of sowing and with temperature and humidity at flowering time. There also was some circumstantial evidence that different parasitic strains of the pathogene may have been present in different years. Durums as a class were more susceptible than common wheats, although there were susceptible and resistant varieties in both groups. No varieties were immune. The following were the most susceptible vulgare wheats: Marquis, C. I. 3641; Kitchner, C. I. 4800, and Red Bobs, C. I. 6255. The following were resistant: Glyndon Fife,

Minn. 163; Haynes Bluestem, Minn. 169; and Prelude, C. I. 4323. Preston, Minn. 924, was resistant for four years, but in 1925 it was heavily infected. Most of the durum varieties were susceptible. Monad, C. I. 3320, and Acme, C. I. 5284, were especially susceptible; while Arnautka, C. I. 1494, and Mindum, Minn. 470, were somewhat resistant. A few durum varieties have always been almost free from infection. Most of the hybrids tested were intermediate in their reaction to scab. Some pure lines obtained from crosses between Marquis×Preston and Marquis×Haynes Bluestem were as resistant as the resistant parents. Some lines of Marquis×Preston appeared to be more resistant than either parent. (Cooperative investigation between the Section of Plant Pathology and the Section of Plant Breeding, Department of Agriculture, University of Minnesota.)

Certain factors influencing the development of the mosaic disease in winter wheat. ROBERT W. WEBB.

Many varieties of winter wheat, notably Currell, show only the mottling phase of mosaic, while only a few varieties, notably Harvest Queen, exhibit both the mottling and rosette phases. Experiments with these two varieties grown continuously in infested soil or transplanted from or to infested soil have shown: (1) that the infection period is confined to the seedling stage; (2) that seedlings transplanted to infested soil appear most susceptible to rosette when four weeks old and less susceptible when younger or older; (3) that under very favorable environmental conditions an exposure to infested soil of one week from date of seeding is sufficient to cause a high percentage of diseased plants, and that longer periods, within limits, give increased percentages; (4) that the disease develops only at relatively low constant or low average soil temperatures; and (5) that the disease development is favored by relatively high constant soil moistures. (Investigations conducted by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.)

The corn mosaic of Hawaii distinct from sugar cane mosaic. L. O. KUNKEL.

Experiments with the corn leafhopper, *Peregrinus maidis* (Ashm.), obtained from North Carolina show that this insect is unable to transmit the virus of the sugar cane mosaic of the United States to corn. A careful comparison made by Mr. Fred Muir, of the Experiment Station of the Hawaiian Sugar Planters' Association, indicates that this insect is identical with the *Peregrinus maidis* of the Hawaiian Islands which transmits the virus of the mosaic disease of corn studied by the writer in Hawaii. This suggests that the destructive mosaic of corn prevalent in Hawaii is distinct from sugar cane mosaic and from the mosaic of corn occurring in Louisiana and other Southern States.

The fungous flora of the nodes of corn. C. L. PORTER.

Isolations from more than 2,000 nodes of corn stalks, collected during a field survey extending over 20 different States during the summer of 1926, showed no specific organism associated with the decomposition of nodal tissues. No correlation between specific organisms and the iron content of the nodes was possible, although as a rule the greater iron content was present in the more highly decomposed tissues. The most prevalent fungi were species of *Fusarium*, *Alternaria*, and *Helminthosporium*. Nine morphologically distinct types of bacteria were isolated. None of these organisms was consistently associated with any type of nodal breakdown. Sound, clean nodes were sterile in many cases.

Smut resistance in corn. MARION A. GRIFFITHS.

Strains of corn, highly resistant to smut under field conditions, were found to be very susceptible to infection when conidial suspensions were injected into meristematic and very young, rapidly growing tissue. The young parts of plants of any age were susceptible. Pouring the inoculum into the top of the plant resulted in a relatively low percentage of infection as compared with the high percentage obtained when the inoculum was injected into the apical bud. Similar results were obtained under field and greenhouse conditions. Resistance of these strains of corn in the field appears due merely to the fact that inoculum does not reach the young growing tissue.

Strains of Ustilago nuda and certain host relationships. W. H. TISDALE and MARION A. GRIFFITHS.

Investigations of the loose smut of barley conducted on Arlington Experiment Farm, Rosslyn, Virginia, show that variants exist within the species *Ustilago nuda* (Jens.) Kell. and Sw. Loose smut collected there in 1923 from Tennessee Winter barley contained two strains of the fungus. Spores from this collection smutted the varieties Han River and Alaska. Spores from Han River smutted Hannchen 100 per cent; while spores from Alaska, which produced 100 per cent smut in Orel, produced none in Hannchen. In no case did smut spores from the Alaska variety smut Hannchen, even though the fungus was grown in other varieties before Hannchen was inoculated. Spores of smut from Hannchen which came through Han River from Tennessee Winter produced 60 per cent of smut in Alaska. Of 32 collections of smut from various sources, 26 produced smut in Tennessee Winter, while only 9 produced smut in Hannchen. Often where smut did not occur in mature plants, infection took place, as evidenced by poor germination, seedling injury, and smutting of secondary shoots from stubble. The amount of seedling injury is not closely correlated with smut percentages.

Cercospora leafspot of Chinese cabbage. W. H. DAVIS.

Leaves of Chinese cabbage (*Brassica pekinensis* Rupr.) bearing paper-white lesions have been under observation for three years in the vegetable gardens at the Massachusetts Agricultural College. The disease was most severe in autumn. Some plants were killed while others were only lightly infected. Circular lesions with paper-white centers crossed by dark-brown veinlets proved unmistakable symptoms of the disease.

An organism, for which the name *Cercospora* (*Cercospora*) *albo-maculans* E. and Ev. is suggested, was found to cause this leafspot.

There are two types of mycelium; hyphae in living tissues are 2μ , in dead tissues 5μ in diameter.

Sclerotia-like bodies giving rise to hyaline conidiophores measuring $3 \times 10-13\mu$ were located in the lesions directly beneath the dead epidermal cells. The conidia are hyaline, elongate-cylindrical, curved, 0-5 septate, $2.5 \times 56\mu$. They germinate with difficulty. The following plants were inoculated with conidia from Chinese cabbage: parsnips, beets, carrots, celery, common cabbage and Chinese cabbage. All except Chinese cabbage remained healthy.

The Fusarium wilt of sweet potatoes on infested soils. R. F. POOLE.

The *Fusaria* which causes sweet potato wilt may be abundant in a field, but seem not to be uniformly distributed through the soil, since some plants remain healthy in infested fields throughout the season, even though many plants are killed in July and August. This was further shown when the percentage of healthy plants was increased

by using two or more plants in a hill. The plants that showed no infection, when harvested in October, were not resistant to the disease, as was shown later by testing them on infested soil. The disease did not spread from one plant to another even when heavily inoculated plants of the susceptible Porto Rico and Yellow Jersey varieties, which became diseased and died, were set one to eight inches from healthy plants of the same varieties. When the soil was artificially inoculated with a heavy suspension of spores the infection was not so severe as that resulting from immersing abscissed and cleanly removed stems in the same inoculum. A large percentage of the plants manifested symptoms of the disease in less than 14 days. Some died within 21 days, while others were not killed until late in October. Likewise, a small percentage of plants inoculated in July showed no symptoms of disease until late September.

Radish black-root caused by Aphanomyces raphani n. sp. JAMES B. KENDRICK.

Cultural and inoculation studies have proved that the causal organism of radish black-root is an undescribed species of *Aphanomyces* for which the name *A. raphani* n. sp. is proposed. *Pythium aphanidermatum* (Edson) Fitz. has not been found associated with the disease, and neither this organism nor *Aphanomyces eutiches* Drechsler have proved pathogenic to radishes in inoculation tests. *A. raphani* resembles *A. eutiches* in its oogonia and oospores, but differs from both *A. eutiches* and *A. laevis* de Bary in that the tapering portions of its zoosporangia from which the zoospores escape show spiral twisting, and in that the diameters of its vegetative hyphae are much greater. The mycelium is intercellular, and the diseased tissues are killed but remain firm unless invaded by rot-producing organisms. Saprophytic bacteria occur very commonly in the diseased tissues. The optimum temperature for the fungus is 23 to 27° C. The disease is more severe on the long than on the round type of radishes owing to the distribution of the infection courts which are the natural wounds made by the emergence of secondary roots from the primary root.

The development of nailhead spot of tomatoes during transit. G. B. RAMSEY and ALICE A. BAILEY.

For many years the disease of tomatoes known as Nailhead Spot has caused considerable damage to the Florida crop in the field as well as during transit and marketing. Lack of data regarding this latter phase of the disease led to a study, during the past two seasons, of the rate of development of the spots present at shipping time and of the development of new spots during transit. Mature green tomatoes were selected at the packing houses and inspected individually. The diameter, in millimeters, of all spots was marked in India ink at the side of each lesion before the fruit was wrapped and packed. The marked crates were shipped to Chicago and a record made of all spots on each fruit at the time of arrival.

The data are summarized in table 1.

TABLE 1.—*The development of nailhead spots of tomatoes during transit*

Diameter of spots, in mm., at shipping point	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Average increase in diameter after 6¼ days in transit.....	1.41	1.22	1.19	1.11	.87	.66	.58	.52	.27	.33	.21

(Contribution from the Research Laboratory on Market Diseases of Fruits and Vegetables, United States Department of Agriculture, Bureau of Plant Industry; the Florida Agricultural Experiment Station, and the Department of Botany, University of Chicago, cooperating.)

Effect of seed treatment on growth, yield, and disease control in vegetable crops. E. E. CLAYTON.

Seed treatment experiments conducted over a three-year period, both in the greenhouse and in the field, have shown disease control effects of two sorts: (1) Seed-borne parasites such as *Phoma lingam* and *Pseudomonas campestris* have been destroyed. (2) Seedlings have been protected during the early stages of growth from attack by soil inhabiting parasites. Under field conditions effects of the first kind have been more frequent.

Stimulation of early plant growth may be readily produced by treating the seed with a variety of chemicals, such as manganese sulfate, arsenic iodide and arsenate of lead. The cucumber has proved very responsive, and striking results have been secured in greenhouse tests. Suitably replicated field tests, however, showed no significant yield differences. In other experiments genuine stimulation effects were apparent. These resulted most frequently from hot seed treatment, and the yields in these cases have been increased as much as 50 per cent. However, it has not yet been found possible to produce these effects consistently.

Retardation of plant growth, aside from reduction in stand, may result from seed treatment. Potatoes soaked in mercuric chloride shortly before planting occasionally showed retarded sprouting and reduced yield. Lima bean seed dusted with a mercury chlorophenolate seed disinfectant regularly produced smaller vines and yields than untreated seed.

*The relation of *Mycosphaerella pinodes* to *Ascochyta blight* of peas.* LEON K. JONES.

Two closely associated species of *Ascochyta*, capable of producing distinct diseases of pea plants, have been found carried frequently in the seed. Pycnospore measurements of the two species are similar and fall within the range given for *Ascochyta pisi* Lib. One organism produces its ascigerous stage on culture media and on infected host parts. Mature perithecia have been found on pea plants 25 days after inoculation and on culture media 15 days after inoculation. Morphological characters of this ascigerous stage are similar to those described for *Mycosphaerella pinodes* (Berk. and Blox.) Stone, but host and cultural relations of the organism differ markedly from those of *A. pisi*. This organism produces a serious brown to black basal stem rot of peas, and indefinite, uniformly purplish brown foliage lesions. The other organism produces host reactions as described for *A. pisi*, but the writer has been unable to find the ascigerous stage of this organism. From a study of the literature and of herbarium specimens at Cornell University, prepared by B. E. Stone, and from inoculation experiments, it is evident that these two organisms have been confused and that *M. pinodes* is not the ascigerous stage of *A. pisi*.

Leaf temperature in relation to tip burn of lettuce. E. L. LECLERG.

The leaf temperature measurements of lettuce indicate no relation between this factor and tip burning. Temperature measurements were made by means of Leeds and Northrup galvanometer reading in eighths of a degree. The lettuce used was grown under glass at known temperature and humidity.

On normal plants in the sun, the top of the leaf was found to be 2° cooler than the bottom 2.1° C. cooler than the surrounding air. In the shade, the top of the leaves was

1.6° C. and the bottom 1.3° C. warmer than the surrounding air. In general the top and bottom of the normal leaves varied but a few tenths of a degree—the upper side in both shade and sun being 0.1° C. to 0.3° C. warmer. The edge of normal leaves was found to be 0.7° to 0.8° C. warmer than the base or main body of the leaf.

Hourly temperature readings, day and night, on lettuce leaves during the process of tip burning showed them to be approximately 5° C. cooler than the surrounding temperature.

Studies of apple scab and cherry leaf spot infection under controlled conditions. G. W. KEITT.

Further studies of apple-scab infection with the same apparatus and method have confirmed and extended the results reported last year (PHYTOPATH. 16: 77). The results then reported on the relations of temperature to infection have not been significantly modified. The minimal numbers of hours of continuous wetting which have yielded leaf infection by ascospores at the temperatures stated are as follows: 6° C., 15; 9°, 11; 15°, 7; 20°, 4; 24°, 6; and 26°, 10. Many ascospores induced infection without developing a well differentiated germ tube, the spore itself functioning as an appressorium from which an infection hypha penetrated directly into the cuticle.

Similar studies of leaf infection of *Prunus cerasus* L. by *Coccomyces hiemalis* Higgins were initiated. Conidia from cultures were suspended in water and applied by atomizers. The initial stages of infection occurred at constant temperatures ranging from 12–13° C. to 28° C. The maximal and minimal temperatures for initiation of infection are not yet clearly established. Lesions developed on inoculated plants incubated at controlled temperatures ranging from 8–11° C. to 28° C. Minimal incubation periods varied from 6 days at 24° C. to 11 days at 28° C. The minimal period of wetting observed to permit infection was five hours at 20° C.

A possible reorientation of aims and methods for apple scab control. G. W. KEITT and E. E. WILSON.

It has been shown by Keitt and Jones that abundant and timely ascosporic inoculum is of primary importance in epidemiology and control of apple scab; and that current fungicidal programs, being adapted primarily to protect fruit, frequently fail to prevent recurrent development of such inoculum. Reduction of ascosporic inoculum may be sought: (1) by preventing foliage infection, (2) by treating living leaves after infection, or (3) by treatment or disposal of dead leaves. Hitherto, considerable attention has been given to the first and third methods, but little to the second. For three years the writers have sought a fungicide which, applied after harvest but before leaf-fall, would economically prevent or limit the development of ascospores without serious host injury. About 75 small-scale trials have been made, chiefly with the following materials: a variety of copper, sulphur, mercury, and arsenic preparations and fluosilicates. These experiments are as yet incomplete and the results inconclusive. However, numerous preparations have appeared to inhibit or prevent production of ascospores. Consequently the post-harvest season prior to leaf-fall appears to cover a potentially vulnerable phase in the life-history of the fungus, and much promise for improvement in the control program seems to lie in sharply limiting the ascosporic inoculum.

Details in the life-history of the apple blotch fungus. EDWIN J. KOHL.

Spores germinated in water on slides adhere closely to the slide and can be washed off only with difficulty. They do not form appressoria on the slide. Spores germinated on leaves form appressoria.

The period of incubation is 20–24 days; on the leaf blade it is shorter than on the petiole. This was determined by placing one-year-old, healthy, potted, Duchess apple trees under a diseased tree, and removing immediately to a safe place after each rain. In 1925, infection occurred at La Fayette, Indiana, during each of the five rain periods between 5 and 8 weeks after petal-fall (May 19). In 1926, infection occurred at La Fayette, Indiana, during 10 out of 24 rain periods between 5 days and 8 weeks after petal-fall (May 18). At Mitchell, Indiana, in 1926, there were 14 rain periods during the 9 weeks subsequent to petal-fall (May 10) and infection occurred only during the 4th and 7th of these periods at 3 and 4½ weeks after petal-fall, respectively.

The mycelium of the fungus has been found to be intercellular and confined to the collenchyma layer. At a considerable distance below the visible margin of the petiole lesion it was traced in this layer into the abscission layer.

Apple blotch canker eradication. MAX W. GARDNER.

The effectiveness of blotch canker eradication in two young apple orchards of the Oldenburg variety at Vincennes, Indiana, has been demonstrated by the freedom of the fruit from infection in blocks of trees left unprotected by the blotch sprays during 1925 and 1926. One of these orchards was set out in 1917, the other in 1918. The eradication campaign was begun in 1922 and consisted in shaving off or pruning out the old cankers and spraying to prevent the formation of new cankers.

Mosaic of red and black cultivated raspberries. W. H. RANKIN.

The degree of control obtained by the use of mosaic-free planting-stock and roguing is extremely variable in New York. Causes of this variation apparently include such factors as: the relative abundance of the principal insect vector, *Amphorophora rubi* (Kaltenbach) Mason; variations in the frequency and intensity of aphid dispersal; and the klendusity of the variety in question. Klendusity (escaping inoculation) and susceptibility are fixed factors in the different varieties and are not correlated. Mosaic is believed to spread almost entirely by the mechanical dispersal of the vector by wind, rain and cultivation operations. A biological relation between the aphid and virus is indicated by the fact that infection in red and black raspberries is initiated only by aphids in the first and second instar.

Mosaic in red and black raspberries has been proved identical and is the most important virus disease of both sorts in New York. The symptoms on black raspberries seem to vary from the initial and progressive necrotic effects to conditions resembling, if not identical with, other previously described virus diseases.

Strains of Pseudomonas tumefaciens. M. K. PATEL.

In isolation studies of *Pseudomonas tumefaciens* from overgrowths on nursery stock and from nursery soils, pathogenic and non-pathogenic strains have been obtained. Fifteen non-pathogenic strains have been recovered in isolations from 200 three-year-old grafted apple trees. Ten of these resemble the pathogenic strain of *Pseudomonas tumefaciens* in morphological characters and in 32 different physiological reactions. The remaining five reacted like the pathogenic strain, except in one characteristic. Of these, four liquefied gelatin and one reduced nitrates. Repeated applications of these strains failed to cause infection with them on tomatoes and castor beans, although trials with the pathogenic strain were always successful. The non-pathogenic strains have been found most abundant in the apple nursery row. They are readily cultured in dilutions on bile agar. The pathogenic strain isolated from soil has retained its pathogenicity for

10 months. The presence of the non-pathogenic strains in overgrowths is probably explained by the presence of the non-pathogenic strain in the soil. Pathogenic strains that have been maintained in culture for two years are still virulent.

Pleospora rot of lemons and apples. D. H. ROSE and L. F. BUTLER.

A species of *Pleospora*, probably *P. herbarum* var. *citrorum*, has been isolated from two lots of decayed lemons from California found on the market. What appears to be the same fungus has been isolated twelve times from apples shipped from Washington, Oregon, and California. The record on lemons confirms an earlier report by Fawcett, although the rot we found occurred on the side of the fruit rather than at the stem end. The record on apples is new as far as we can discover.

Cross inoculations prove that cultures from both lemons and apples are pathogenic to both kinds of fruit and suggest that the fungus making the initial attack is the same in both cases.

On both lemons and apples the rot develops slowly; affected tissues are brown to almost black, firm and rather dry. In lemons the attack is confined usually to the peel; in apples it may penetrate to the core.

The fungus produces both ascospores and conidia (*Macrosporium*) in culture. Single-spore cultures from these have in every case reproduced both kinds of spores. This seems to prove that the conidial stage of this species of *Pleospora* is a *Macrosporium*.

The effect of spraying with fungicides on the keeping quality of Florida citrus fruits.

HARRY R. FULTON and JOHN J. BOWMAN.

Florida citrus fruits are liable to losses in marketing from two types of blue-mold rot caused by *Penicillium digitatum* and by *P. italicum*, and also from two types of stem-end rot caused by *Phomopsis citri* (*Diaporthe citri* Wolf) and by *Diplodia natalensis*. Extensive experiments for six seasons show that a single application made April 15 and May 5 of 3-3-50 Bordeaux mixture plus 1 per cent oil in the form of an emulsion will reduce total rot about one-third during a prolonged marketing period. The effect is mainly on the more common *Phomopsis* type of stem-end rot, which is reduced more than one-half. The *Diplodia* stem-end rot is reduced only about one-fifth by the one application. *Penicillium* rots are not materially affected. This application is the one used for controlling the melanose blemish caused by the same species of *Phomopsis* and can be expected to give about 90 per cent melanose control. Two spray applications in the spring control rot slightly better in the marketed fruit than one application, owing mainly to increased effectiveness against the *Diplodia* rot. Mid-summer and fall applications are relatively ineffective. The oil emulsion is necessary in the Bordeaux mixture and also must be used alone a few weeks later to check increase of scale insects following the destructive action of the copper fungicide on the entomogenous fungi which keep these pests in check.

Watermelon wilt infection studies. D. R. PORTER.

In 4,700 isolations from watermelon vines infected with *Fusarium niveum* Smith, this fungus has been obtained from stems, leaves, petioles, pedicels, fruit, primary, secondary, and tertiary roots and probably seeds.

This *Fusarium* causes cankers on the primary and secondary roots at any point below the surface of the soil. They are, however, more prevalent from 6 inches to 2 feet down, and appear in early stages as water-logged, light yellow lesions, varying in

length from one-half to several centimeters. Vascular invasion follows the development of lesions, which, in early stages, is local rather than general. The organism may then spread very rapidly throughout the root system. Maximum wilting occurred in 1926 after the melons had set.

Supposedly immune citron vines have yielded *Fusarium nivewum* pathogenic to watermelon seedlings. Seemingly healthy watermelon plants, on land that had never before produced watermelons, likewise yielded the fungus. The fungus appears to remain alive in soil where watermelons have not been grown for at least 16 years.

Bacterial halo spot of kudzu. FLORENCE HEDGES.

Bacterial halo spot of kudzu is of particular interest because of the steadily increasing economic importance of kudzu in the south as a forage plant and a cover crop for pecans. The wide yellow halo surrounding the small brown spots is strikingly like that in tobacco wildfire. When the spots are close together, the halo is less evident or disappears altogether. Young lesions are angular and water-soaked. The disease has been reported by Clinton in Connecticut and Boyd in Georgia. In a survey of kudzu fields in Georgia and Florida, the writer found the disease in new fields planted with roots from a diseased stand, while other fields in the vicinity were free from infection.

From the lesions, the writer has isolated a polar-flagellate, non-spore-forming, aerobic, white organism pathogenic both to kudzu and lima bean. On beef agar it forms bluish white, somewhat opalescent, crenate colonies with internal cross-hatching; it liquefies gelatin, produces acid from glucose and saccharose in beef extract agar, but none from lactose; slow peptonization, but no acid or reduction of litmus, produced in litmus milk; no reduction of nitrates; blue-green fluorescence in Fermi and Ushinsky; nutrient gelatin greened, beef broth and beef agar slightly so; feeble diastasic action; steamed-potato cylinders grayed; cream colored growth on potato. The organism has been tentatively named *Bacterium pueriae*.

A mosaic resistant variety of cucumbers. O. H. ELMER.

A variety of cucumbers named "Chinese Long" obtained from Professor R. H. Porter, of Nanking, China, in tests during the past year has proved highly resistant to the cucumber mosaic disease. Porter stated concerning it that "Last year I did not observe any (mosaic) in this locality—(nor)—thus far this season." Chinese Long cucumber is a slicing type not suitable for pickling.

Mosaic inoculation tests conducted in the greenhouse, where 50 plants each of the varieties Chinese Long and White Spine were inoculated, have to date resulted in no infection in Chinese Long and approximately 100 per cent infection in White Spine. In a field plot consisting of five six-rod rows, aphids transferred from mosaic cantaloupe plants failed to induce infection.

Curly-top of squash. M. B. MCKAY and T. P. DYKSTRA.

In August of this year, a severe disease, never before observed on squash, was found in many places in Oregon, Washington and Idaho. Generally the plants appeared yellow and badly stunted, the leaves had yellow, upturned margins and were small, though in some varieties the margins turned down and the leaf blades were crinkled. Often the plants produced a new runner from the axil of each leaf on the main branches. Some tissues in the roots were noticeably discolored, being yellow to light brown in color. Plants affected early remained very small and developed a striking witches'-broom type of growth. The disease was observed on the following varieties or kinds of squash:

Boston Marrow, Green Hubbard, Summer Crookneck, Mammoth White Bush Scallop and Silver Skin. The general failure of squash in the Northwest this year was due largely to this disease.

Circumstantial evidence suggested that the disease was due to the virus of the sugar beet curly-top disease. The disease has been readily and repeatedly produced in the greenhouse by the use of beet curly-top viruliferous leafhoppers, *Eutettix tenella*, on healthy plants. Individual leafhoppers which induced curly-top on squash also caused curly-top on sugar beet. Untreated controls invariably remained healthy. (Cooperative investigation between the Oregon Agricultural Experiment Station and the Bureau of Plant Industry—Office of Vegetable and Forage Diseases.)

Commercial tobaccos and cured leaf as sources of tobacco mosaic. W. D. VALLEAU and E. M. JOHNSON.

Results of inoculation of a number of commercial brands of tobacco and air-cured leaf of different sources and ages indicate that these may be sources of the mosaic disease in tobacco. The disease has been produced by inoculations of tobacco from four brands of plug, three brands of cigarettes, and five brands of granulated smoking tobacco. Almost 100 per cent infection has been produced from air-cured leaf as old as five years, both in field and greenhouse tests. Field tests indicate that primary infection from the use of tobacco by workmen in the field can be reduced to less than one-half of one per cent (in one test to zero) by requiring the use of heat sterilized tobacco by the workmen while pulling plants from the bed and during transplanting in the field. Preliminary experiments indicate that the disease can be produced from dried tobacco as old as 18 to 30 years.

*Correlation of virulence and acid agglutination of a smooth and a rough strain of *Bacterium phaseoli sojense*.* C. G. SHARP.

A smooth and a rough strain, which have remained constant in culture, were isolated from a culture of *Bacterium phaseoli sojense*.

Smoothness apparently is correlated with greater virulence, and roughness with lesser virulence. When inoculated into leaves of soy beans by needle pricks, the smooth strain produced 62 per cent infection in 4 days; the rough, 39 per cent. Both strains produced 100 per cent infection in 8 to 10 days. The lesions produced by the smooth strain averaged 1.033 mm. in diameter (575 measurements). Those produced by the rough averaged 0.808 mm. (579 measurements). By passage through plants, the rough strain has been converted to the smooth.

Roughness and lesser virulence are correlated with greater agglutinability. The rough strain agglutinates spontaneously in distilled water. The smooth one does not. The rough strain agglutinates in distilled water plus acid or alkali through the wide range of pH 1.2 to 9.4 with low agglutination at about pH 5.5. The smooth one agglutinates only through the narrow range pH 2 to 3.

The organism of the smooth strain is very motile. The rough one is either very sluggish or non-motile.

These strains cannot be differentiated serologically (agglutination and precipitin tests).

The results of these investigations demonstrate concordance between phenomena in plants and those previously reported in animal pathology.

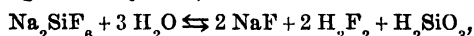
Correction of unproductive muck by the addition of copper. E. L. FELIX.

Certain more or less sharply defined areas of the muck soils in Western New York, involving approximately 400 acres, have been found unproductive, especially for lettuce

and onions. Plants grow normally for a time and then suddenly assume pronounced abnormalities which result in complete failures on severely affected soils. Experiments of the past three seasons show that the most prevalent type of unproductivity can be corrected by the addition of copper to the soil or plants. Applications of pulverized copper sulfate crystals at the rate of 100 to 200 pounds to the acre, prior to sowing, resulted in the production of normal crops. Painting the leaves of affected lettuce with a weak solution of copper sulfate caused the plants to outgrow the abnormalities. Dusting lettuce grown on unproductive muck with "20-80 copper-lime dust at the rate of 55 pounds to the acre likewise caused the plants to become healthy. Experiments indicate that copper is the beneficial element added and that it may be a vital factor in the growth of plants on such soils.

A theory to account for the bactericidal action of sodium silicofluoride and lack of injury to host tissues. H. W. ANDERSON.

A 1-200 solution of sodium silicofluoride used as a spray on peach trees has successfully controlled bacterial spot, caused by *Bacterium pruni*, without serious injury to the host tissues. According to the equation,



hydrolysis would result in the formation of hydrofluoric acid, supposedly highly toxic to plant tissues. H-ion determinations of dilute solutions (1-100,000) show that complete hydrolysis and dissociation occur, but in more concentrated solutions (1-1,000) only partial dissociation occurs. When sodium silicofluoride solutions are sprayed on foliage the water evaporates, leaving the salt. When water is added, sodium silicofluoride should be present in the following conditions: (1) undissolved; (2) dissolved and not hydrolyzed; (3) dissolved and hydrolyzed but HF not dissociated; (4) dissolved and HF dissociated. The first three conditions probably are predominant and, while the amount of dissociated HF on the leaf at one time is not sufficient to cause injury, yet a 1-25,000 solution of sodium silicofluoride will kill *B. pruni* in 10 minutes. A 1-50,000 solution will kill over 99 per cent of them. Hydrofluoric and hydrochloric acids in proportionate strengths are equally toxic to *B. pruni*. Hard water used in mixing the sprays reduces the acidity of the sodium silicofluoride solution, but not enough to interfere with the efficacy of the sprays.

Fungicidal control of brown-patch of turf. JOHN MONTEITH, JR. and T. CARTER HARMON.

Mercuric fungicides again proved most effective in controlling brown-patch (*Rhizoctonia* spp.) of turf. The organic preparations (Uspulun, Semesan, Germisan, Corona—620, Corona—640) were effective against both the common types of disease. Adjacent plots were treated with mercuric chloride, sulphate, sulphide (black), oxide (red) and cyanide; mercurous chloride and nitrate; Uspulun; and Semesan. Each plot received an equal weight of metallic mercury. Results indicated that control was largely dependent on the amount of mercury present. Only the sulphide was ineffective. This probably was due to its insolubility in the soil acids. Injury to grass depends on the chemical combination, mercuric cyanide being most toxic and mercurous chloride least toxic. From the standpoint of control, toxicity, and cost, the most satisfactory chemical was mercurous chloride. Less than one-fifth pound of this compound proved fully as effective as one pound of Uspulun or Semesan, which cost approximately 10 times as much. Silver nitrate gave control similar to that of the mercury salts. Repeated applications of copper (copper sulphate, Bordeaux, copper stearate) resulted in an accumulation of copper which proved extremely toxic to the roots. Sulphur sprays or dusts likewise are injurious.

Formaldehyde, super-kalimat, phenol and sodium silicofluoride proved too toxic when used in amounts sufficient to control brown-patch.

The permeability of the seed coat of corn to mercury compounds. C. R. ORTON.

Permeability studies were conducted with four sweet and three dent varieties of corn using mercuric chloride (1:1666), chlor-phenol-mercury sulphate (1:400), nitro-phenol-mercury sulphate (1:400) and cyan-cresol mercury (1:400).

Uniform seeds were soaked in distilled water. Then the part of the seed coat covering the embryo and that covering the endosperm were removed and tested separately. They then were dried and cemented to the ground ends of glass tubing of uniform diameter and placed in sterile water for 24 hours. All leaking and imperfectly cemented seed coats were discarded.

The perfect seed coats were tested by placing two cubic centimeters of distilled water inside each tube. The tubes were then suspended in a mercuric solution of known concentration. Mercury in the distilled water inside the tube was detected by the sodium-hydrosulphite test.

Apparently that portion of the seed coat covering the embryo side of the seed is more slowly permeable than that covering the endosperm. It appears also that the rate of permeability of seed coats to each mercury compound varies with the corn varieties.

The four compounds naturally fall into three classes according to the approximate rate at which they pass through corn seed coats. Mercuric chloride and cyan-cresol-mercury pass through in approximately 30 minutes or less; nitro-phenol-mercury in an average of about 60 minutes; and chlor-phenol-mercury (Uspulun) requires an average of approximately 80 to 145 minutes.

Tree injection for control of fungous diseases and insect pests. C. M. SCHERER.

During the summer of 1926 American chestnut (*Castanea dentata*), white birch (*Betula alba*), gray birch (*Betula populifolia*), apple (*Malus* sp.), and American elm (*Ulmus americana*) were injected with various chemicals for the purpose of controlling chestnut blight (*Endothia parasitica*), bronze birch borer (*Agrilus anxius*), and European elm scale (*Gossyparia spuria*), respectively.

The chestnut trees were injected with acid fuchsin, acriflavine, aniline blue, basic fuchsin, Bayer Compound, brilliant green, ferrous sulphate, gentian violet, lithium carbonate, malachite green, methyl blue, safranin, Semesan, sodium carbonate, sodium fluoride, Sulfocide, thymol, zinc chloride, and Zonite; the birches with arsenic trioxide, Bayer Compound, Black-leaf 40, and strychnine sulphate; the apple with acid fuchsin, gentian violet, and thymol; and the elm with strychnine sulphate. These experiments included 85 chestnut, 75 birch, 22 apple, and 2 elm trees. The trees ranged from 1¼ inches to approximately 24 inches in diameter.

The smallest amount of any injected material was ½ gram of arsenic trioxide in a 1¼-inch birch, and the largest amount was 1,045 grams of strychnine sulphate in a 23-inch birch.

All results were negative with the exception of the apple trees which were injected with thymol. These seemed to show a definite resistance to the progress of *Baotilus amylovorus*.

Soil treatments for the control of damping-off in coniferous seed-beds. J. STEWART WIAINT.

Experiments to determine the value of soil-treatments with certain chemicals for the control of damping-off in coniferous seed-beds, begun in 1925 at Ithaca, New York,

were continued in 1926 at Keene, New Hampshire. *Rhizoctonia solani* Kühn was the principal pathogene involved.

Effective control was secured in beds of *Pinus strobus* and *Picea excelsa* with chlorophenol-mercury (Semesan and Uspulun), mercuric chloride, and nitrophenol-mercury (Bayer Compound). In beds of *Pinus resinosa*, satisfactory results were obtained with aluminum sulphate—a fungicide recommended by Hartley and his associates, Uspulun, mercuric chloride, Bayer Compound, and sulphuric acid. The average damping-off in untreated beds of these three species was 43.5, 23, and 30.9 per cent, respectively. Under the conditions of these experiments, the above chemicals cannot be differentiated on the basis of their effectiveness as control agents.

Three organic mercury dusts furnished by the Bayer Company under the numbers 1-8, 101 and 112 gave significant increases over untreated beds in final stand of *Pinus resinosa* and to a somewhat lesser extent indicated value in beds of *P. sylvestris* and *Picea excelsa*. Copper carbonate, applied in powdered form at the rate of four to eight grams per square foot, produced severe chemical injury.

Sulphur and copper carbonate dusts as efficient fungicides for the control of sorghum kernel smut and millet smut. L. E. MELCHERS and C. O. JOHNSTON.

Fungicidal dust treatments will replace liquid methods for the control of kernel smut of sorghums and millet smut. Experimental results at the Kansas Agricultural Experiment Station have proved the effectiveness of copper carbonate for sorghum smut control. This treatment is widely prevalent in Kansas and is being introduced into other sorghum-growing states. Two years' results have proved the copper-carbonate treatment the most practicable and efficient method of millet-smut control. The same applications are used as for wheat smut, but the 50-55 per cent grades of copper carbonate are given preference.

Four seasons' results from extensive plantings of smutted sorghum seed treated with flowers of sulphur and the more reduced sulphur dusts, such as "Sulfodust" and "Kolo-dust," indicate a control of sorghum kernel smut equal to that obtained with copper carbonate. The cost of treatment is about one-sixth of one cent an acre. This includes labor, cost of sulphur and investment in equipment. The various sulphur dusts do not control millet smut satisfactorily. (Cooperative investigations by the Kansas Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

The x-bodies in the cells of "mosaic diseased" and "dwarfed" dahlias. BESSIE GOLDSTEIN.

Intracellular bodies similar to the x-bodies of mosaiced tobacco, corn, *Hippeastrum*, and wheat have been found in the cells of dahlia plants affected with two types of disease—"mosaic" and "dwarf." They occur in all the tissues of young leaf primordia, growing points, and blotched leaves, though they are not present in yellow-margined leaves so far as studied. These intracellular bodies give very clear evidence that they are not mere reaction products of the cell protoplasm. They show a definite indication of flowing and elongation movements in the form of pseudopod-like extensions of the body surface. Structures resembling nuclei and vacuoles are present. They are found in all stages of what appears to be division by constriction, including such interesting forms as those in which the two divided halves of the body proper have become rounded up within a clear space, the stretched and constricting portion of what appears to be a membrane remaining still unbroken between them.

Cryptoporus volvatus and its relations with forest trees and insects. A. H. REGINALD BULLER.

Dendroctonus beetles and their allies, by means of their tunnels, prepare the way for the inoculation of coniferous trees with *Cryptoporus* spores and facilitate the production of *Cryptoporus* fruit-bodies on the exterior of the bark.

The fruit-bodies of *Cryptoporus volvatus* are unique among the Polyporeae in having their hymenial tubes contained in a chamber instead of being freely exposed to the air. The basal volva is perforated by an ostiole. Beetles and other insects enter the chamber and carry away spores which have settled on the volva. It is probable that certain of the spore-laden insects play an important rôle in the dissemination of the fungus. Some of the spores probably escape via the ostiole and are carried off by the wind. Fruit-bodies are sometimes formed at the mouths of entrance or exit tunnels of Ambrosia beetles, but there is no reason to suppose that these beetles cultivate *C. volvatus*.

Physiology and parasitism of Sclerotium rolfsii. B. B. HIGGINS.

The scope of the investigation includes: (1) temperature relations, (2) relation of reaction of the substratum to growth, (3) reaction changes in the substratum during growth, (4) metabolic products on various media, (5) relation of the metabolic products to parasitism, (6) relation of fungous hyphae to the host tissue.

The minimum, optimum, and maximum temperatures for growth are 8°, 30–35°, and 40° C., respectively. The maximum temperature for continued normal growth of the fungus appears to be about 37° C. Freezing kills the vegetative mycelium but the sclerotia withstand a temperature of –10° C. for 48 hours.

The fungus grows on beef-extract-peptone broth from pH 1.4 to 8.3. Addition of 1 per cent NaCl to broth shortens both the alkaline and acid range. Addition of 2 per cent saccharose extends the alkaline range to pH 8.8 but shortens the acid range. The reaction of broth is changed by growth of the fungus toward a fairly definite end point near pH 4.0. In broth plus a carbohydrate there is no definite end point, but the change is always toward the acid side.

In beef-extract broth the principal products of growth are ammonia and oxalic acid with traces of succinic and formic acids. In broth plus a carbohydrate, less ammonia and more oxalic acid are produced. The amount of free oxalic acid is sufficient to kill the hypocotyl of tomato and soybean seedlings. The mycelium grows over the surface of a plant and kills the outer cells before entering the tissue. Oxalic acid may be readily demonstrated in the dead cells. Later the hyphae enter and produce a soft rot.

Serological differentiation of Bacterium campestre from *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*. GEO. K. K. LINK and C. G. SHARP.

Bacterium campestre, *Bact. phaseoli*, *Bact. flaccumfaciens* and *Bact. phaseoli sojense* are yellow schizomycetes which in culture are quite similar morphologically and physiologically.

By serological methods it was attempted to determine whether *Bacterium campestre* (the crucifer pathogen) could be differentiated from the three yellow pathogens of beans, and to determine its relationship to them. Agglutination tests were applied to these organisms. Serum was prepared by injecting rabbits intravenously with live organisms, and bleeding them 10 days after the last injection.

In low dilutions, the writers obtained group agglutination with antisera of the bean organisms against *Bacterium campestre*, but did not obtain agglutination in any dilution with the anti-serum of *Bact. campestre* against *Bact. phaseoli*, *Bact. phaseoli sojense* and

Bact. flaccumfaciens. The anti-serum of *Bact. campestre* agglutinated *Bact. campestre* in dilution up to 1-7680.

On the basis of current interpretation, these results mean that *Bacterium campestre* contains no protein capable of stimulating production of an antibody which will react with *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*, but that the bean pathogens contain proteins which cause production of antibodies that do react with *Bact. campestre*. Thus, *Bact. campestre* can be differentiated serologically from *Bact. phaseoli*, *Bact. phaseoli sojense* and *Bact. flaccumfaciens*. This means that these organisms, though closely related, are serologically distinct.

Serological and physiological studies of Bacterium phaseoli, Bact. phaseoli sojense, and Bact. flaccumfaciens. C. G. SHARP.

Agglutination tests have been applied to *Bacterium phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*. At first, serum was prepared by injecting rabbits interperitoneally with dead organisms and later intravenously with living ones. The rabbits were bled 10 days after the last injection. Later it was found that serum can be prepared by beginning injection intravenously with living organisms. Specific agglutination was obtained with each organism against its homologous serum in dilutions up to 320; no agglutination was obtained with heterologous sera. The results indicate that these organisms contain serologically different proteins, since they can be differentiated by agglutination tests.

Agglutination tests were supplemented by growing the organisms in 2 per cent sugar broths sterilized by filtration and adjusted to pH 6.8 to 7.2. In ten days, and longer, *Bacterium flaccumfaciens* formed sufficient acid in dextrose, galactose, levulose, lactose, and maltose to differentiate it from the other two organisms. In 30 days *Bact. phaseoli* and *Bact. phaseoli sojense* produced little change in dextrose, maltose and sucrose, but formed acid in galactose. In 30 days *Bact. phaseoli* changed levulose from pH 6.8 to pH 6.4-6.8 and lactose from pH 7.1 to pH 7.6. *Bact. phaseoli sojense* changed levulose to pH 7.3 and lactose to pH 8.2. The latter organism produces a characteristic surface growth and precipitate in lactose and levulose.

Formal titration of gelatin cultures of *Bact. phaseoli* and *Bact. phaseoli sojense* will not serve to differentiate between these organisms, but will differentiate *Bact. flaccumfaciens* from the others.

Pythium ultimum and *Pythium debaryanum*. CHARLES DRECHSLER.

Of the species of *Pythium* encountered in pathological relationships and assigned indiscriminately to *Pythium debaryanum* Hesse, the one most frequently concerned in damping-off, stem-rot, root-rot and rootlet injury is identical evidently with the forms discussed morphologically under this binomial by DeBary, Ward, and at least in part by Miyake. As pointed out by the writer in a note on *Pythium* infection of cabbage heads, it apparently never exhibits the condition figured by Hesse, i.e., a longish antheridial branch arising from the hypha bearing the oogonium at a considerable distance from the latter. As the fungus is undoubtedly identical with *P. ultimum*, described by Trow as a saprophyte though presumably on inadequate evidence, it should be known by this binomial.

A fungus of which the usual relation between oogonium and antheridium approximates that represented in Hesse's figures is also frequently concerned in damping-off, root-rot and rootlet decay. Cylindrical-stalk antheridia and pouch-like antheridia arising in immediate proximity to the oogonium are decidedly rare. The oospore-wall is thinner than that of *Pythium ultimum* ($1.0 \pm \mu$ instead of $1.5 \pm \mu$), the "reserve glo-

bule'' larger, the strongly flattened refringent body oblate ellipsoidal rather than sub-spherical. Zoospores, never observed in *P. ultimum*, are here produced in moderate abundance. To this form, the oogonia and oospores of which do not differ greatly from those of *P. ultimum* in size (measuring respectively $22 \pm \mu$ and $18 \pm \mu$ in diameter in both species), the binomial *P. debaryanum* can more correctly be applied.

A peculiar type of Pythium. CHARLES DRECHSLER.

Three related species of peculiar pythiaceous fungi were isolated in 1926 from diseased stems and rootlets of various hosts including the bean, peony, ragweed and touch-me-not. The sporangia are terminal, often proliferous in moderate measure, and normally, as in *Pythium*, deliver their undifferentiated contents through an evacuation-tube of variable length into a vesicle where the zoospores are formed. Unlike *Phytophthora*, they do not discharge fully developed zoospores from a sessile papilla. In the sexual apparatus, features departing from the types more usually distinctive of *Pythium* are found in the elongated branch antheridium, roughly grub-like in shape, being closely applied to the oogonium along practically its entire length; in the fertilization-tube arising from a navel position on the antheridium; in the hyphal elements bearing the sexual organs exhibiting helicoid involvement regularly in one species, and more rarely in the other two; in the unusually thick-walled ($3 \pm \mu$) mature oospores containing approximately 15 to 20 vacuoles or "reserve globules," and 5 to 10 smaller refringent bodies presumably to be interpreted as nuclei, instead of the single reserve globule and single refringent body present in the generality of forms usually assigned to the genus.

Relation of storage temperature to lag in growth of fungous cultures. D. H. ROSE and L. F. BUTLER.

When petri-dish cultures of *Physalospora rhodina* (Berk. & Curt.) Cooke and *Pleospora* sp. are withdrawn from the temperatures at which they have been held for six to eight days and placed at room temperature for two days longer, there is a lag in growth which is correlated with, and apparently due to, the temperatures at which they had previously been held. Cultures held at the four temperatures 3, 7, 11, and 13° C., with the variations in each case rarely more than $\pm 0.5^\circ$ C., were studied. Immediately after removal from the temperature cabinets, the plates were spread in a single layer on a table and left two hours. Thermometer tests indicated that the plates all came to the room temperature within 80 minutes after being spread out. At the end of the two hours the plates were stacked and covered with cotton laboratory aprons. The results of the tests are given in table 1.

TABLE 1.—Average increase in diameter of colonies upon removal from various storage temperatures to room temperature

Name of fungus	No. of experiments	Hours exposure at room temperature	Av. increase in diameter of colonies (centimeters)			
			Previous temperatures			
			13° C.	11° C.	7° C.	3° C.
<i>Physalospora</i>	4	24	1.71	1.18	0.76	0.58
<i>Pleospora</i>	2	24	0.69	0.67	0.50	0.50
<i>Physalospora</i>	4	48	3.61	3.13	2.71	2.65
<i>Pleospora</i>	2	48	1.03	1.25	0.94	0.93

The effect of different hosts upon some species of Phytophthora. LEON H. LEONIAN.

The size and shape of *Phytophthora* sporangia constitute the most important characteristics in a strictly morphological classification. The followers of such a scheme must, of necessity, assume that the species is an immutable entity. The writer has shown that environmental conditions exert a tremendous influence upon the morphological features of *Phytophthora* spp. Since a host plant offers as many diverse physical and chemical factors as any laboratory medium or reagent, it is only natural to expect that different hosts exercise different influences upon the same species of fungus. With this point in view, the writer inoculated green tomatoes, green peppers, egg plants and potatoes with a number of *Phytophthora* spp. Ready infection and abundant fruiting followed. Sporangia from different hosts showed such striking dissimilarities in size and shape as to fool the most conservative morphologist.

The natural conclusion, therefore, is that a purely morphological system of classification is not of great value, and that only the more constant morphological features should be combined with reliable physiological reactions if a more satisfactory scheme of classification is desired.

Variation of strains of Alternaria solani isolated from lesions on potato tubers. REINER BONDE.

Alternaria solani cultures isolated from lesions on potato tubers were found to comprise distinct strains, which can be differentiated on the basis of formation of pigment in potato agar, rate of growth, macroscopic appearance of mycelium in culture, production of conidia, and pathogenicity on leaves and tubers. Saltations occur rather frequently in some strains, giving rise to new forms distinctly different from the parent. Some of the strains studied arose by saltation occurring *in vitro*. The strains cannot now be differentiated by measurements of the conidia.

The red pigment of the chromogenic strains was intensified by high temperatures and was retarded or absent at lower temperatures. Differences in acidity and alkalinity of the medium did not suppress pigmentation in the more chromogenic strains. Some intermediate strains were non-chromogenic in media of high acidity. Sunlight intensified the pigmentation of certain strains. Other strains remained non-chromogenic under all conditions of environment to which they were subjected. The pigment formed by *Alternaria solani* is deep red or carmine when alkaline, and yellow when acid.

The chromogenic character of the strains in media is not correlated with pathogenicity.

Clitocybe root-rot of trees and other woody plants in Florida. ARTHUR S. RHOADS.

During the past three years the writer has observed, at various points in Florida, several cases of mushroom root-rot of trees and other woody plants caused by *Clitocybe tabescens* (Scop.) Bres., which he previously reported as the cause of a root-rot of grapevines in Missouri (Jour. Agr. Res. 30: 341-364. 1924). *Clitocybe* root-rot has been observed most frequently in guava (*Psidium guajava*) trees, killing them in old citrus groves at Courtenay and Lotus on Merritts Island and at Cocoa, Rockledge, Bonavenura, Mt. Dora, and Astor. Additional instances of equal severity have been observed in the case of eucalyptus trees at Cocoa, Okeechobee, Orlando, and Sebring; Australian pine (*Casuarina equisetifolia*) at Okeechobee and Rockledge; rose apple (*Caryophyllus jambos*) at Ft. Pierce, and poinsettia (*Euphorbia pulcherrima*) at Oakland.

Additional study may demonstrate that *Clitocybe* root-rot of various trees and other woody plants is of fairly widespread occurrence throughout Florida and other southern

states. Despite the frequently noted occurrence of *Clitocybe* root-rot in guava trees of long-established citrus groves, no instance has been found where citrus trees have been attacked.

A study of the downy mildew, Sclerospora graminicola (Sacc.) Schroet. I. E. MELHUS, FRANK VAN HALTERN, and D. E. BLISS.

A completely saturated atmosphere, turgid leaves covered with a film of moisture, and a temperature between 10° and 27° C. seem to favor sporulation of *Sclerospora graminicola* on *Setaria viridis* (L.) Beauv. Light apparently is not a factor. Under favorable conditions, sporulation occurs within 4 to 6 hours, and germination in water occurs in one hour at temperatures between 5° and 20° C. The optimum temperature is 14° to 18° C. The sporangia are forcibly discharged vertically 1.5 to 2.5 mm. and laterally 1.44 to 1.89 mm. *Sclerospora* has been reported on *Setaria glauca* several times, but it has never been collected by the authors, although the two species of *Setaria* regularly occur together in fields in Iowa and adjoining States.

Sclerospora hibernates by means of oospores rather than by intraseminal mycelium. The oospores require no rest period and, in dry conditions, remain viable at least 17 months. The minimum period of incubation following sporangial infection of corn by either oosporic or sporangial conidia is 6 days. Infection may take place near the base of the plumule 24 hours after the plumule emerges from the seed coat. Such infection generally is systemic. On corn, 11 per cent infection resulted from sporangia, and 91 per cent from oospores. The oospores were not killed after soaking 10 minutes in a 2 per cent copper-sulfate solution, nor when heated at 50° C. for one hour. Five minutes in one per cent formaldehyde inhibited oospore germination, while one-tenth per cent mercuric chloride for 40 minutes at room temperatures did not.

Some effects of mosaic on the content of the cell. MELVILLE T. COOK.

The cells of a series of leaves from the center to the outside from both mosaic and apparently healthy plants of sugar cane and tobacco have been studied for the purpose of determining the effects of mosaic. The chloroplasts in a mosaic plant are smaller and fewer than those in a healthy plant, but they tend to become normal with age. That is, the chloroplasts in the outside leaves of a mosaic plant are very nearly the same in appearance as those of a healthy plant. These studies indicate that the chloroplasts are not destroyed nor even injured by the disease. Their development, however, is inhibited. The nuclei of many mosaic cells are enlarged and otherwise deformed but they tend also to become normal with age. Islands of diseased cells are to be found in apparently healthy tissues, and islands of healthy cells in diseased tissues. There was no indication of recovery from the disease. Intracellular bodies were commonly found in diseased tobacco but rarely in diseased cane.

Separation of fern leaf from mottling in tomato mosaic. SOPHIA H. ECKERSON and H. R. KRAYBILL.

Juice from mosaic tomato plants was filtered through sintered glass filters having pores about 4 microns in diameter and through collodion membranes. By means of the glass filter it was possible to retain on the filter all of the infectious principle which produces mottling symptoms and to recover in the filtrate substances which produce fern-leaf and filiform symptoms in tomato plants similar to those frequently associated with tomato mosaic. If the tomato juice was allowed to stand in an ice box and then centrifuged, the juice when filtered through the glass filter would produce mottling symptoms. None

of the filtrates from collodion membranes produced typical mottling symptoms but some produced the fern-leaf and filiform symptoms. Attempts to infect other plants with the juice of plants showing only the fern-leaf symptoms were negative. Thus juice from the mosaic tomato plants was separated into two fractions, the residue containing the infectious principle (probably an organism) which produces typical tomato mosaic, and the filtrate, a non-infectious principle (possibly of the nature of toxins) which passes through collodion membranes and produces only fern-leaf and filiform symptoms with no mottling.

Multiplication of the virus of tobacco mosaic in detached leaves. HELEN A. PURDY.

The virus of tobacco mosaic, when inoculated into detached tobacco leaves, produces no macroscopic symptoms of disease but causes the production of intracellular bodies similar to those associated with tobacco mosaic in the leaves of growing plants. The appearance of these bodies after a definite incubation period and their occurrence in tissues distant from the point of inoculation indicates a development of the virus in the cells of the detached leaves.

Hoping to obtain further evidence of multiplication of the virus in cells of detached leaves, serial transmission experiments were undertaken. A known amount of virus was introduced into the first leaf of each series. After a period of incubation the sap was extracted from the leaf, diluted with distilled water and a given amount inoculated into a second detached leaf. In this manner, each leaf of a series was inoculated successively. The dilution of the original inoculum transferred was estimated in every case. The dilution used in the inoculation of the ninth leaf, juice from which produced mosaic in healthy plants, is estimated to be not less than 256×10^{-18} in each series. This is many millions of times the water dilution necessary to inactivate the virus and indicates multiplication in the detached leaves.

Leafhopper injury to clover. E. A. HALLOWELL, JOHN MONTEITH, JR., and W. P. FLINT.

For several years an injury resembling tipburn or hopperburn of potatoes has been observed on clovers and other legumes. It was conspicuously prevalent and widespread during the summer of 1926, and was associated with an unusual abundance of leafhoppers. At Urbana, Illinois, red clover was grown in adjacent insect-proof cages, one heavily infected with leafhoppers (*Empoasca mali*), the other free from insects. These tests apparently demonstrated that leafhoppers were responsible for the condition observed, and further showed that the relatively glabrous English clover suffered more than the pubescent Tennessee strain. Affected leaves, sometimes simply yellowed or bronzed, are often slightly curled and usually show tip and marginal browning, while frequently the entire leaf turns brown. The plants remain dwarfed, and many die. As on potatoes, Bordeaux appeared to repel the leafhoppers, and the plants remained vigorous. On red clover at Arlington Farm, Virginia, where numerous species of leafhoppers were abundant, the damage was most noticeable on Italian clover, while the native hairy strains showed much less injury. Zigzag, alsike, and white clovers showed similar symptoms in varying degree. Alfalfa in Italian clover plots, as well as in extensive plantings nearby, was likewise severely dwarfed and had the typical "yellows" or "yellow-top" appearance.

Investigations on citrus "blight," wilt or leaf-curl in Florida. ARTHUR S. RHOADS.

During the past three years, an intensive study has been made of a chronic wilt of citrus trees, locally called "blight," or leaf-curl, that has caused heavy losses in groves of certain sections of Florida during the past 40 years. Extensive experiments in bud-

ding young trees and grafting older ones demonstrated that this trouble is not transmissible. No evidence was obtained to indicate that a pathogenic organism is responsible for this trouble. It is believed to be of non-parasitic origin, and traceable to extremes in soil-moisture conditions. There is a well-defined relation between the occurrence of citrus "blight" and the water-holding capacity of the soil type. This trouble appears to be caused most frequently by an inadequate supply of soil moisture during the dry season of the year. Often it may be due also to excessive amounts of soil moisture resulting from prolonged saturation of poorly drained grove areas. Again, it may be intensified by the recurrence of drouth following an excessively wet season. Citrus "blight" clearly is greatly favored by neglectful care of the grove. After the trees develop the chronic wilt, control appears possible only through adoption of preventive rather than remedial measures.

A serious disease of birches. PERLEY SPAULDING.

There has appeared within a few years a serious disease of various birches in our eastern forests. In 1919 A. H. Graves published concerning a *Nectria* canker of *Betula lenta* L. and still earlier Pollock mentioned a *Nectria* canker of *B. lutea* Michx. During the summer of 1926 a similar disease was found apparently epidemic on *B. lutea* in Vermont and on *B. lenta* in Massachusetts. There is much diversity in the symptoms, but after studying them for some days the writer came to the conclusion that he also was dealing with a disease caused by *Nectria*. Foresters have been alarmed during the last few years by an increasing decrepit condition of yellow birch and have attributed it to the bronze birch borer. The trouble found by the writer seems not to be due to an insect though the borer is not excluded as a cause of serious injury. Cultures are being made for further work. While the disease is not generally serious, it may be well to have it in mind so that its range, virulence and economic importance can be learned. Any notes concerning it will be much appreciated.

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SUGAR CANE ROOT DISEASE IN CUBA: A PROGRESS REPORT UPON THE ROOT DISEASE SITUATION IN 1925¹

J. A. FARIS AND R. V. ALLISON

There has been much concern expressed by the cane growers of Cuba over what has been referred to for some time as "root disease." In the field it appears that this term has been widely applied to cane dying under a great variety of conditions, in fact under practically any condition that does not manifestly involve the above-ground parts of the plant structure. This tendency to bring every malady affecting the under-ground parts of the plant under the general term of "root disease" or "root rot" has been confusing and in many cases has resulted in planters throwing land out of cane over considerable areas because of a disease about which they know



FIG. 1. Successive stages in the death of cane affected with "root disease."

¹ Scientific Contributions No. 4, Tropical Plant Research Foundation. From the Cuba Sugar Club Experiment Station, Central Baraguá, Cuba.

nothing. However, it now seems that the disease in many of these areas is due to one or more of the soil conditions discussed in the second part of this report, and in several cases studies are being instituted by the planters to reclaim some of this land.

Investigations have been in progress in many of the more important cane-growing countries for many years, and everywhere the studies have been uniform and emphatic in pointing out the necessity for proper agricultural conditions for growing cane. In many cases what has appeared to be a serious disease menace has disappeared when some adjustment has been made in the matter of soil drainage, fertility, or cultivation. Owing to the confusion created by referring to such a large group of primary causes of disease as root disease, it seems desirable to briefly review the situation as we have found it at the end of our first year's work, with the hope that the



FIG. 2. Longitudinal section of the base of a cane stalk, showing characteristic foot rot due to *Melanconium*.

planters will study the failing areas in their fields in the light of this discussion.

In order to analyze the root disease situation and to facilitate the location of the initial weakening of the plants, the primary causes of the death of the cane are considered as due in part to pathogenic factors and in part to non-pathogenic factors.

The possible pathogenic invading organisms require further study for a determination of their nature, habits, and probable spread, but cannot be said, in the light of our present information, to be very serious factors upon any of the considerable number of plantations visited.

POSSIBLE PATHOGENIC CAUSES OF ROOT DISEASE

The work upon this group of possible causes consists in the isolation of probable primary invading organisms and determination of their capacities for infecting healthy plants.

Symptoms

Plants dying of this complex malady exhibit certain rather marked symptoms. The outer leaves first show signs of drying and rolling up, while the inner leaves are still green. This is followed by rolling of the inner leaves and death of the outer ones. This drying may continue until all of the leaves are dry and the plant dies. In figure 1 is a series from a single hill which shows a gradual progress from a slightly affected stalk on the right (stalk 1) to one with leaves completely dry (stalk 4). The root systems of these stalks were poorly developed and the bases of the stalks themselves were found to be badly infected with the *Melanconium* species later discussed. Such plants are not uncommon in many of the fields of Crystallina cane in Cuba. These drying out changes may be brought about by any cause which cuts off the water supply from the top of the plant. In various parts of the Island, during both the wet and dry seasons, fields have been observed which contained plants in all stages of drying out. Usually the trouble is more conspicuous during the dry season, and any obstruction of the water flow would be reflected in the lack of moisture delivered to the growing parts at that time.

From the study of diseased cane plants in certain areas where decreasing production was attributed to root disease, it became evident that a very large number of these plants not only had diseased roots but also had developed what might well be called a foot rot (at the bases of the stalks). Plants affected with this basal stalk rot have a zone of dying tissue extending from the base of the plant upward. In some cases it is this progressing zone of diseased tissue which cuts off the water supply from the top of the plant, even though the root system is quite well enough developed to supply the needed moisture. There are several types of this basal stalk rot, at least one of which shows many indications that it is primarily due to an invading parasitic organism. This type of foot rot is described as zonate foot rot in another article.² The type under discussion here, pictured in figures 2 and 3, is associated with species of the fungus *Melanconium*, which proceeds from the base upward in the stalk, with an advanced zone of infected bundles, usually of a blackish color, extending through several internodes of the stalk. Figure 2 shows longitudinal sections of the base of an infected cane stalk with the blackened zone extending up one side. Figure 3 shows the brownish woody stem bases in which the water-conducting strands have been plugged up, thus preventing the functioning of the root system.

Isolations

As soon as the first two months' preliminary survey of the cane root disease situation in Cuba was finished, intensive work was begun upon the

² FARIS, J. A. Zonate foot rot of sugar cane. PHYTOPATH. 17: 83-94. 1927.

fungi associated with these root and stalk rots. Isolations were made of a very large number of fungi from diseased tissues. After study and identification, many of these were eliminated as probable causes of the malady upon the grounds that they had been repeatedly experimented with by other investigators and found to be harmless to healthy cane. Among the fungi found in these tissues were two species of *Melanconium*; one of these, *M. iliau*, which causes the iliau disease of sugar cane in Hawaii, had not been previously reported from Cuba. It seemed quite probable that this might be an active parasite infecting the cane plants, although its destructiveness in the Hawaiian fields is attributed by Lyon to the tight binding of the leaf sheath of the young plants, thus preventing the development of the young shoots. The other, *M. sacchari*, as is well known, causes the ever-present rind disease. In view of its prevalence in these diseased plants, it seemed worth while to make some inoculations with pure cultures of this fungus and determine its pathogenicity. Since it was desired to follow the development of the root disease more closely, further experiments were carried out in order to insure infection in case neither of the *Melanconiums* proved to be capable of infecting the healthy plants. In these tests the seed piece was



FIG. 3. Bases of cane stalks infected with *Melanconium*.

covered with an abundance of finely chopped roots and stalk bases of diseased plants.

In connection with these experiments several different soils were used in order to determine, if possible, the influence of soil type upon the development of the disease. The soils were taken from spots where plants were dying from the disease. These soils were placed in sacks $2\frac{1}{2}$ feet long and about 8 inches in diameter. One portion of the soil was sterilized. Lengthwise through the center of the sacks which were to be sterilized was placed a piece of rolled screen. This was done to facilitate sterilization but proved unnecessary, as perfect circulation of the steam was secured without it. Sterilization was done under six pounds steam pressure for three hours. One series of plants was grown in the soil without treatment, and the other series was planted in the same soil sterilized with steam.



FIG. 4. 1. Plants inoculated with *Melanconium iliau*. 2. Check, uninoculated. Soil I, sterilized. No infection.

Method of Inoculation

In the case of *M. iliau* and *M. sacchari*, the fungi were isolated in cane plug cultures, cornmeal agar, and cane juice agar, and these pure cultures multiplied in flasks. From these flasks blocks of one cc. were taken, placed upon a stick of Crystallina cane which had been sterilized in mercuric bichloride (1 to 1000) and rinsed in distilled water, and held in place by a piece of filter paper. Each seed piece had three nodes, but the two end buds

were removed when the center one was inoculated. These seed pieces were then planted in pots which were made by cutting gasoline cans in halves.

Soil Types Used .

Four soil types were used in these experiments, and have been referred to by number in the tables. Type I (Havana clay) represents a heavy, ashy-gray to black, calcareous clay that passes down through calcareous clay of a lighter color into cream-colored chalk at an average depth of ten or twelve inches. In places, light-colored, slabby limestone occurs at shallow depth and not infrequently is strewn over the surface in considerable quantity, particularly where the topography is rolling. Where it is not too shallow, this type ranks very high as a cane soil, giving good yields and high sugar return under good cultivation. Type II, a deep red soil (Matanzas clay), derived from "dog-tooth" limestone such as is found in large areas in the provinces of Havana and Matanzas, is also an excellent cane soil. Type III (Oriente clay) is a very heavy black soil which, characteristically, is immediately underlain by cocó. This is ranked as a rather poor cane soil. Type IV (Limones clay) is a heavy, purplish-red clay, apparently formed

TABLE 1.—The effect of inoculating *Crystallina cane* with *Melanconium iliau*. Cane planted March 6, 1925, and harvested January 1, 1926

Type of soil and treatment	Inoculations	No. of plants	Results
I. Sterilized	Inoculated	56	Free from root and stalk rots
do	Uninoculated	6	do
Unsterilized	Inoculated	6	do
do	Uninoculated	6	do
II. Sterilized	Inoculated	9	do
do	Uninoculated	9	do
Unsterilized	Inoculated	9	do
do	Uninoculated	9	do
III. Sterilized	Inoculated	9	do
do	Uninoculated	4	do
Unsterilized	Inoculated	4	do
do	Uninoculated	4	do

from the breaking down of serpentine rock. In this the topsoil, upon drying, breaks into hard clods. This passes into pinkish red to red clay at 4-6 inches and into parent material (undecomposed or partially decomposed serpentine and other rock material) at 1-4 feet. It is only a fair cane soil but can be brought to fairly high production by good cultivation and the application of cachaza (filter-press cake).

Results with Melanconium iliau

In no case did *M. iliau* prove to be capable of infecting the growing cane plants, even when the organism was put over the buds at planting time. It should be pointed out that this is but one of the organisms found associated with root and stalk rot in the field. The plants were kept growing vigorously, as will be seen from the size of those in figure 4. A portion of the plants which had been inoculated with *M. iliau* was cut to the ground surface and these plants will be carried to the first ratoon. The pots are very small and the soil is filled with a dense mat of roots, so the plants are developing against great odds.

Results with Melanconium sacchari

A second series of experiments was carried out in a similar way with the rind disease fungus, *M. sacchari*, which was always associated with the foot rot of a seedling cane. The fungus was isolated from blocks of diseased tissue, increased in pure cultures in cane juice agar, and blocks of the actively growing fungus were placed over the young cane buds at the time of planting. Only one soil type was used in the experiment, results of which are summarized in table 2.

Here again the results indicate that this fungus is not able to infect healthy, vigorously growing Crystallina cane. Figure 5 shows some of the plants in this series.

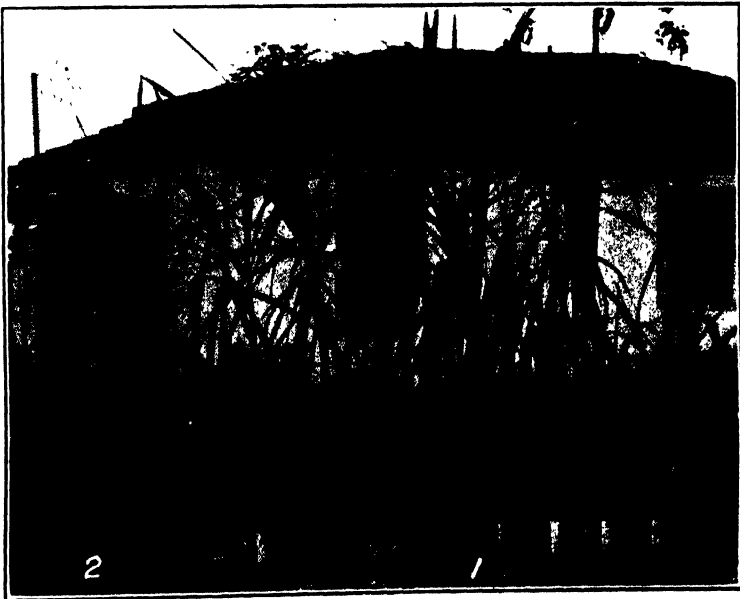


FIG. 5. 1. Plants inoculated with *Melanconium sacchari*. 2. Check in sterilized soil.

TABLE 2.—*The effect of inoculating Crystallina cane with Melanconium sacchari. Cane planted March 23, 1925, and harvested January 7, 1926*

Type of soil and treatment	Inoculations	No. of plants	Results
I. Sterilized	Inoculated	24	No sign of root or stalk rot in any of the plants do do do
I. Sterilized	Uninoculated	4	
I. Unsterilized	Inoculated	6	
I. Unsterilized	Uninoculated	6	

Inoculation Experiments with Various Organisms

In this series of experiments the bases of ratoon *Crystallina* plants dying from root disease were chopped into small pieces and the material used to inoculate the seed pieces. The buds were covered with a quantity of the chopped material, which was held in place by moist filter paper. The soils were taken from diseased areas where considerable complaint of losses from root disease had been made. Soils I and IV were used in this series and were treated as in the previous experiments. The results are summarized in table 3.

Here, again, when diseased tissue was used for inoculations so that any parasitic fungus present might have opportunity to act, no signs of the root disease developed (Fig. 6). The plants were removed from the small cans,

TABLE 3.—*The effect of inoculating Crystallina cane with chopped diseased cane roots and stalk bases. Cane planted March 23, 1925, and harvested January 8, 1926*

Type of soil and treatment	Inoculations	No. of plants	Results
I. Sterilized	Inoculated	18	No sign of infection do do do do do
I. do	Uninoculated	4	
IV. do	Inoculated	8	
IV. do	Uninoculated	3	
IV. Unsterilized	Inoculated	6	

the soil washed away from the bases of the stalks, and a careful inspection made of both the roots and the stalks. The latter were split down to the seed piece. The soil was washed from the roots of typical plants from some of these experiments. The root systems of these plants are shown in figures 7 and 8.

Discussion of Results

Since the type of root disease under experimentation in these three series of cultures is that characteristic of a very large percentage of the troubles of

this nature all over the Island, and since the preliminary experiments failed to show any primary invading organism, we must analyze the problem somewhat further to see if we can arrive at the key to the situation. It is evident that we are dealing with a variety of cane-growing conditions, but in the end the invading organisms, with some exceptions, are the same. These organisms we find to be semi-parasitic in that they invaded only weakened tissues and thus were not capable of infecting the healthy plants in the experiments. Therefore it becomes evident that in all of these soils we have some factors operating to injure or weaken the plants. Field studies in various parts of the Island have indicated some of these causes contributing to the dying out of the cane, the more important of which are discussed later. The term root rot, which has been so commonly applied to this dying out of cane, covers a very large number of primary causes for the weakening of the plants, which are then invaded by fungi which could not otherwise attack them. In realization of this fact, which has been established by experimentation and field observation, the first thing to be investigated when plants in the field begin dying out is whether the trouble may not be accounted for by one of the reasons discussed below. We do have some cases which cannot be thus accounted for. These are under investigation, but the experiments have not progressed far enough to be reported upon except to describe the occurrence of the disease, its effect upon the plants, etc.



FIG. 6. 1. Plants inoculated with material obtained from chopped diseased cane roots and stalks. 2. Check, uninoculated.

NON-PATHOGENIC CAUSES OF ROOT DISEASE

Under non-pathogenic factors will be grouped a number of conditions, essentially ecological, which are of direct concern in determining the physical and chemical fitness of the soil environment to the health and development of the roots of the cane plants. While any of the factors mentioned below may be individually active and are discussed more or less individually, it is seen that in a given case in nature a number of them may be simultaneously involved in the development of a given condition in the soil which, for several reasons, may be extremely deleterious to plant growth. Furthermore, as will also be discussed, some of these factors may be active in a more or less alternate fashion and in this way insure a more continuous injury to the health of the plants. This might well be instanced in cases where the soil is especially subject to conditions of flood and drought. Thus an intricate complex series of factors frequently must be taken into account, as well as the individual conditions referred to, in considering the health and development of the cane plant in any particular case. The enumeration and discussion of the more outstanding of these conditions which so frequently constitute the primary cause of the so-called root disease in cane represents the purpose of this part of the report.

Lack of Drainage

In point of area concerned, lack of or deficient drainage is one of the most important reasons why cane can not be grown to any extent in the Island.

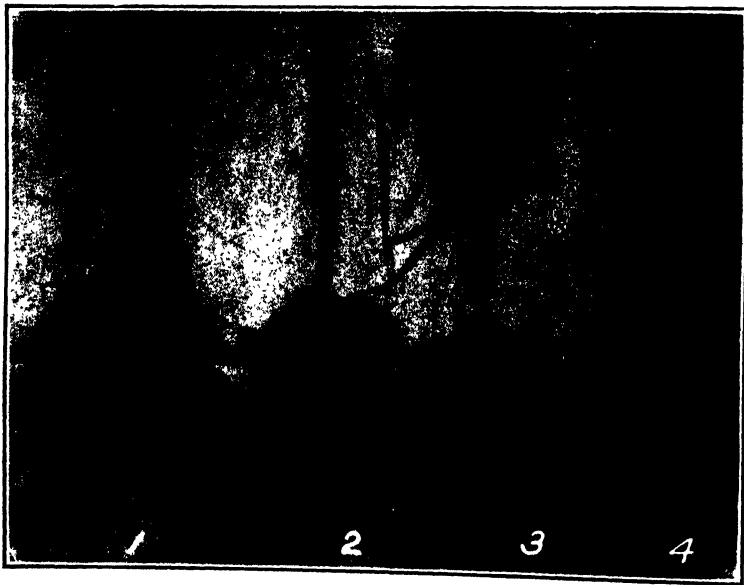


FIG. 7. Root systems of plants artificially inoculated. 1. Soil III, inoculated with *Melanconium ilia*. 2. Soil II, inoculated with *Melanconium sacchari*. 3. Inoculated with chopped diseased cane roots and stalks. 4. Uninoculated check.

This condition is particularly prominent in the regions of the heavy brown and black impervious soils of the coastal plains and river flats throughout the country, especially in the Province of Oriente.

In our observations, extensive areas are encountered very commonly where the question of economic production upon the soil involved has absolutely no basis for discussion or consideration until an efficient system of drainage is supplied.

In picturing a plant struggling along in such an environment it must be remembered that every living cell in its tissues is entirely dependent upon the life-sustaining process of respiration. This is dependent, in turn, upon ready accessibility to air. Imagine the anaemic nature of the process in one of these suffering plants with its roots buried in sticky, soggy clay, where at times the whole of the soil surface is flooded with free water through periods perhaps of several days or weeks. Plants dying under these conditions of strangulation have been frequently referred to as victims of root rot.

It has been found that the drainage problem is further complicated over rather extensive areas, particularly in Oriente and upon the Coastal Plains in the other provinces, through the presence of common salt in toxic concentration. In certain areas adjacent to or recently influenced by the sea in this and other provinces, the impregnation of these heavy clays with salt is so great that it frequently appears as surface incrustations upon the banks of



FIG. 8. Root systems of plants artificially inoculated. 1 and 2. Soil IV inoculated and uninoculated with chopped diseased cane roots and stalks. 3. Soil II inoculated with *Melanconium iliau*.

shallow ditches or on the bottoms of evaporating pools. Samples of the soils have been analyzed from these general regions which show a total salinity as high as 0.3, 0.4, and even 0.5 per cent. The concentration is usually found to increase with depth. In terms of total salts the incrustations referred to as being left by small evaporating pools contain as much as 5 per cent as sampled. Salt flats in positions adjacent to the sea and bearing characteristic vegetation in the form of shrubs, grasses, and weeds quite commonly have a salt concentration in the top soil of 2 per cent. While this is manifestly not a cane soil, we have seen cane planted in such positions in actual competition with mangrove and salt willow.

As indicating the rather general condition in this connection, the concentrations given in table 4 were found at the depths indicated in a fairly heavy coastal clay in the south of Havana Province. Here the surface soil was a dark brown clay, passing at two inches into a lighter brown plastic clay, and at 5 inches into yellow plastic clay. This became heavier and more impervious with depth, and a faint gray mottling appeared at about 44 inches. These samples were taken early in 1925 and the analyses made by the Bureau of Soils in Washington.

The present discussion is concerned primarily with such concentrations of salts as will visibly affect the health of the plant through toxicity to grow-

TABLE 4.—*The concentration of salt at certain depths in a heavy coastal clay soil in southern Havana Province, Cuba*

Sample no.	Soil depth in inches	Salt in per cent
32659	0- 2	0.21
32661	5- 8	0.41
32662	8-44	0.67
32664	56-65	1.04

ing roots. Injury from such a source of course predisposes these organs to natural rotting. Active toxicity of this type has been observed in several areas. In the case of the cane plant, however, it is highly important to understand that the tonnage of the crop removed from saline soils gives no assurance of a normal rendement of sugar in the mill. For, while the salt may be present in sub-toxic concentration in the soil and the plants develop in an apparently normal manner, its accumulation in their juices may still be such as to hinder the crystallization of sugar in the mill, and a high production of molasses will result.

Similarly, waters from the ditches or wells of some of these regions are heavily charged with salt and frequently brackish. Waters from open ditches have shown as high as 11,000 parts per million in terms of total salts. While on these areas the absolute drainage conditions may vary somewhat, either locally or in general, from those discussed above where the

salt factor is absent, the solution for their development is absolutely the same, namely, drainage. For unless ditches are opened for the natural washing out of these readily soluble salts, the situation may be expected to become increasingly serious with continued cultivation. Under such conditions, the plants, with the natural handicap of the presence in solution of a toxic substance, usually make less growth or perish more quickly than where deficient drainage alone is a factor.

Besides the direct effects of these drainage conditions upon the plant, their damage is reflected indirectly through the effect of the changed physical structure and aerability of the soil mass upon the development and activity of the soil microflora. This, as noted, is produced through the lack of aeration brought about by the general degeneration of the physical condition of the soil structure as well as the presence in certain areas of the toxic salts indicated. The depression, or loss in this manner, of the activity of the various important groups of the soil flora in their effective preparation of certain of the soil constituents for assimilation by plants must also be regarded as a serious matter.

Lack of Moisture

In contrast to the conditions discussed under lack of drainage, moisture deficiencies are usually most commonly associated with the pervious red soils so far as the soil types of importance in cane production are concerned. To be sure, serious difficulties of this nature are also experienced in the lighter sabana soils when they are cultivated. This is due largely to the fact of their extreme permeability and comparatively small capacity for retention of available moisture. The moisture situation in this respect is complicated by the alternation, in seasons of approximately six months each, of rainfall and drought. Thus the cane upon soils that are weak in their ability to store water may naturally be expected to suffer greatly during this extensive period of rainfall deficiency. Furthermore, certain types of red soil appear to dry out much more than others. Thus in February of last year, 1925, in certain areas of a red soil in Camagüey, no apparent moisture was encountered even at a depth of 40 inches. The soil was so dry that it could be brought to the surface, even from this depth, only with great difficulty, since it powdered under the soil auger and fell back into the hole as the instrument was withdrawn. This has since been found to be the usual condition of this soil during the dry season.

Upon a soil of this type the moisture deficiency referred to is regarded as of first importance, and the protection of the plant in this respect, particularly by methods of planting and subsequent cultivation and fertilization, is a most important consideration.

Moisture deficiencies also appear during the dry season upon the heavy brown and black soils, referred to previously as usually suffering from lack

of drainage. Here the results are frequently very serious; in contrast to the general deportment of the red soils, the brown and black soils usually crack very badly. This, in addition to producing serious physical damage to the roots of the plants, exposes a much greater surface of the soil, and particularly the deeper layers, to evaporation. The desirability of preventing this tendency both for the protection of the roots of the plants from physical injury and desiccation and the conservation of moisture in the soil is readily apparent and will be discussed further.

In either case the weakness imposed upon the roots of the plants by such a long period of desiccation is a very serious handicap to their immediate and future development. The mutilation of these organs in the fashion indicated also leads to an undue exposure of these parts to the natural microflora of the soil, which is ever seeking ready sources of energy of the type represented in abundance by the carbohydrates in these structures. Consequently, if the conditions are too rigorous, the roots are certain to decay.

There are extensive areas in the western part of the Island where this condition is to be very commonly observed, either in patches or in whole fields. Here the stand of cane exhibits varying degrees of degeneration. It is a noteworthy fact, and one that has been demonstrated in several instances, that where these areas have been cultivated and planted according to practices in keeping with rational agriculture, the trouble disappears entirely and good yields result. It is a fact truly worth keeping in mind that the manipulation of the available supply of moisture is a most important consideration in the cultivation of cane in Cuba. However, where plants have been overcome by this general condition, and their entire root system practically destroyed by the drying out and cracking of the soil, the trouble is frequently referred to as root rot.

Deficient Cultivation

The term cultivation is here used to include practices in the preparation of the seed bed, the planting of the seed, and the treatment of the soil subsequent to the appearance of the young plants, whether plant cane or ratoon, previous to the closing of the middles.

It is obvious that cultivation in this sense is very intimately related to the conditions of drainage and moisture discussed above. In the case of heavy impervious soils the chief significance lies in the matter of plowing in banks and level planting, while in the instance of the pervious red soils it is deep plowing and deep planting. Observations have been made upon plantings under good conditions of cultivation in both cases, however, which indicate the desirability of some very fundamental studies in root range, particularly in relation to varietal adaptability before too specific recommendations can be made in this connection.



FIG. 9. Roots of cane, showing the effect of planting and cultivation on root development. The white line indicates the original position of the soil level. The figures on the tape indicate decimeters.

Whether upon porous red soil or the other extremity, the impervious brown and black clays, proper cultivation or other practices productive of the same results subsequent to the appearance of the young plants is an absolute necessity in the control of grass and weeds. Herein is one of the great dangers of deficient drainage, for the excess of moisture which promotes the growth of obnoxious weeds in the cane fields simultaneously prevents the application of such cultural practices as will not only destroy this growth but assist very materially in the maintenance of a proper physical condition of the soil.

In the majority of cases this can best be done by moving the trash to alternate middles, or, where the trash is light, from two middles into a third, and cultivating those exposed. By such a procedure a sufficient trash covering is put upon one row to keep down the weeds, while in those exposed they are destroyed by cultivation. In the case of the heavy, stiff, black clays, this procedure serves not only to destroy the weeds but to reduce the drying out and cracking of the surface and sub-surface soils and consequently effectively to prevent the physical injury to the roots which these conditions produce. It was largely through the development of such a pro-

gram of cultivation that the successful restoration of the declining production on the red soils of western Cuba was first locally demonstrated.

Figure 9 shows the difference to be expected between poorly planted and cultivated canes and those receiving the treatment described. The stool on the left represents primavera (spring) cane at one year of age. This was planted in April, 1924. The cane piece shown in cross section in the central part of the root mass represents the original seed piece. Two months before the picture was taken, when the cane was less than 10 months old, a test cutting showed a yield of 97,000 arrobas of net cane per caballeria (37 short tons per acre). The roots on the right represent one of the best stools in the first ratoon of an improperly planted and improperly cared for field. Here at the first cut from primavera the yield was slightly less than 25,000 arrobas per caballeria (9.5 tons per acre). At the time of taking the picture the stand in the latter case was showing all the conventional signs of root rot, being very irregular and having many patches and areas from which the cane had practically disappeared. Taken as a whole the crop was scarcely worth cutting. At the time of harvest the weight of net cane in the former stool was 84½ lbs., while that of the inferior stool was 5 lbs.

In matters of cultivation, the manner of cutting the cane at the time of harvest and the method of applying fertilizers are of considerable importance in relation to the control of the depth of root development. This is made apparent by the illustrations in figure 10. The stools were taken from a burned-over area on an average red soil in Camagüey Province, and show the extreme superficiality of the root systems that may develop after a succession of annual cuttings. These stools were removed after the eighth cutting from cane that had not been cultivated since planting in virgin soil after burning the original forest growth. The tendency is especially well shown in B, where the buds that germinated were exceedingly superficial and the general condition and position of the root system in relation to the surface of the soil (indicated by the white line) is very bad.

Attention is called to the shallow dead stems on the left in figure 10, A, with the rootstock passing from beneath these to the right. It is seen that the maximum depth of this part is slightly more than 4 inches. There were a total of four plants in this stool. Two other stems sprang from the upper part of the rootstock, but were cut away to show the connections more clearly. The shallowness of the prospective root system is apparent. The general condition in B is particularly bad in this respect. Note that the stalks on the left (two smaller ones cut away from front) spring from top of old stem to right, also the larger stems to the right. This old stem is seen to be rotting down the center to within an inch of the dead rhizome of the year past. Parts of the lower section of the young plants were also becoming discolored at the time of examination. It is a point of importance in connection with the plants here discussed that in the general area of red

soil from which they were taken, infestation by root mealybug is particularly common and frequently bad. In the immediate area from which these stools were taken an examination of the roots of more than 50 stools showed an infestation of 100 per cent.

The tendency for the stool to rise, as referred to above, may also be accentuated by fertilizing methods. In the application of the fertilizer materials it is recognized as the better procedure in the case of ratoon cane to make a *desaporque* (furrow turned from along rows toward middle) along both rows in the exposed middle and spread the fertilizer along the exposed bases of the cane stools. After this application the soil should be worked back into the furrow by subsequent cultivation. The importance of the offbarring in cutting away the old root structures and aerating the soil mass about the living centers of the cane stool is readily apparent and has long since been established in Cuban agriculture.

The application of the fertilizer in the bottom of such furrows rather than on the top of the stool is to be no less emphasized. By so doing the material is placed in a position to be immediately available to the newly



FIG. 10. Photographs A and B showing the superficial nature of roots of ratoon plants taken, after eighth cutting, from burned over area on red soil, Aug. 19, 1925.

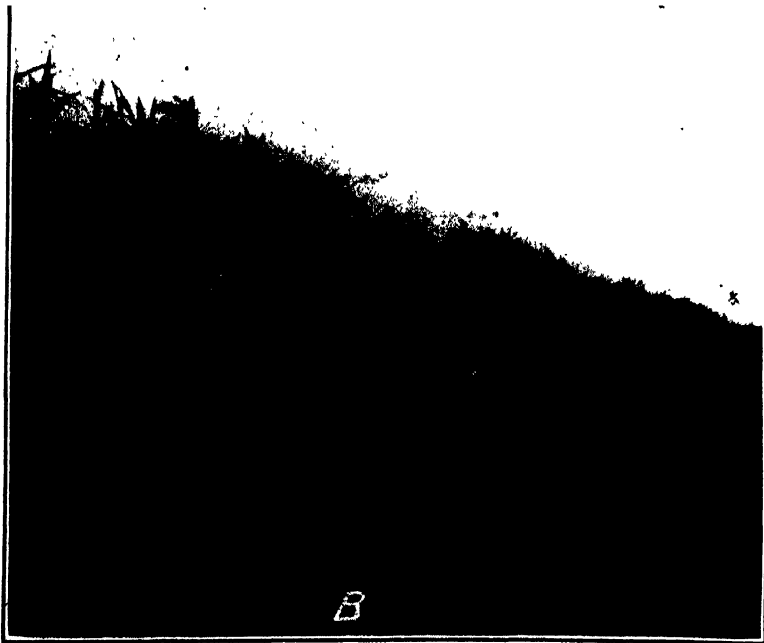
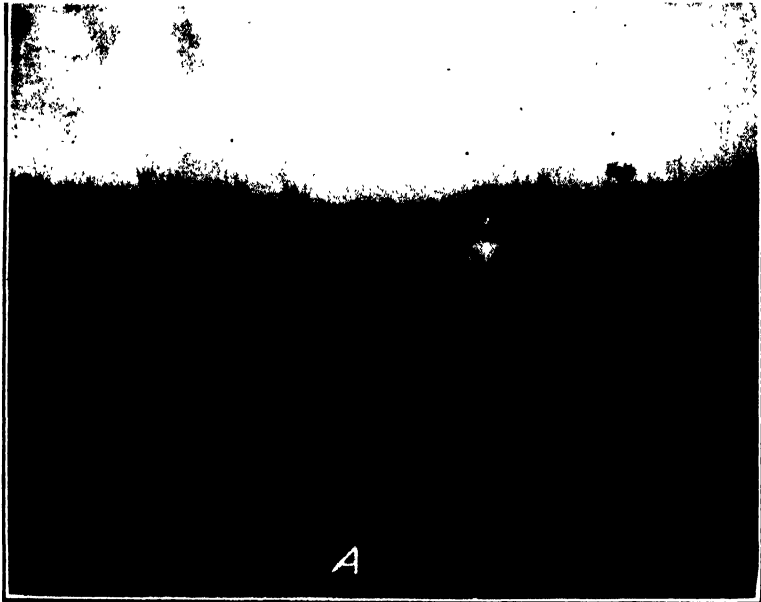


FIG. 11. Cane growing upon good red soil in Matanzas Province on opposite sides of same guardarraya, although on different colonias. A. No fertilization, no cultivation; B. fertilized and cultivated.

formed roots of the plant. By virtue of its position, the tendency also will be for their development downward rather than upward, as might be expected where it is placed superficially above their normal zone of growth.

The general degeneration in the stand and yield of cane as the result of deficient cultivation is well shown in figure 11, A and B. These two fields are located upon opposite sides of the same guardarraya, and the soil, identical in both cases, is of the good deep red Matanzas clay type derived from limestone. These fields, though adjacent, are upon different colonias, and the tremendous difference in the cane stand is due to fertilization and cultivation. The grass-ridden cane shown in A is not an instance of root disease or root rot, as frequently has been inferred under similar circumstances. It is simply an excellent though unprofitable demonstration of very poor farming, where this condition develops as a consequence of the fact that the preparation of the soil, the planting of the seed, the application of fertilizer, and consequent cultivation of the cane has been in entire disregard of the factors here discussed.

Deficient Fertility

It is generally understood that deficiencies in soil fertility in Cuba are more likely to be encountered in the older agricultural areas of the West. It is not to be doubted, however, that careful studies in relation to soil type will indicate specific deficiencies of a more or less local nature to be generally distributed throughout the Island. Since outstanding deficiencies in any of the more important elements necessary for plant growth are known to be the reason for a greatly reduced vigor and resistance, disturbances of the nature generally discussed above must be expected to appear when the soil solution to which the plant has access becomes unbalanced in this respect and one or more of the elements becomes unavailable.

The reduction of the vigor of the plants, and consequently the severity of attack of the fungi and bacteria of the soil upon the weakened roots, will thus be more or less directly determined by the extent of the deficiency involved. Deficiencies of this nature, particularly in relation to phosphoric acid and potash, have been studied in connection with both corn and cane for several years. In fact, it is now definitely thought that the so-called resistance to these conditions among certain varieties is nothing more nor less than a lower requirement for the element of which other susceptible varieties find an inadequate supply in the same environment. This may be due either to natural differences in feeding power for the same materials or to actual differences in requirements. The latter appears the more plausible as between varieties.

While the outstanding elements which the plant must draw from the soil for the elaboration of its food are nitrogen, phosphorus, and potassium, it may happen that still others are present in deficient quantities. They may

also be present in such unbalanced proportions as to develop either a toxic condition or one that is suboptimum to the assimilative interests of the plant in one way or another. Thus it is possible that in some soil types there may be a positive calcium deficiency. In others the presence of an excess of magnesium over calcium may repress the activity or availability of an otherwise abundant supply of this element even though it be present. This latter condition seems to be the case most frequently in soils derived from serpentine and is well displayed upon *bibijagua* (leaf cutting ants) mounds, where the toxicity, apparently due to the material brought up from the subsoil, persists even after the mound has almost disappeared as a result of cultivation. In certain of these soils the Ca/Mg ratio has been found to be 1:3 or even wider; in fact, comparatively high concentrations of magnesium have been found along with but a trace of calcium. A further consideration will be given this matter in connection with the various types derived from this material as well as with other types when more analyses are available. On the other hand, where the soil is deficient in calcium and the material brought up by these insects is calcareous, the benefits to plant growth are at once apparent in the better growth and color of the cane upon these spots. This contrast of effects is commonly to be observed in one way or another upon soils of this type. In the case of those having the injurious type of subsoil referred to, the natural outcrop of this material is frequently the cause of local to fairly extensive *sabana* areas.

Manifestly the only logical solution of this general phase of the problem is a systematic study of varietal requirements in relation to soil type. At the present time, however, upon areas where the cane is doubtless suffering from specific deficiencies of this nature, in complex with one or more of the conditions discussed above, the impaired stand and dying cane is sometimes attributed to root rot.

Other Factors

In a substratum possessing such native complexity as the soil, there is a great variety of factors that might appear to disturb the metabolism of living plants which are directly dependent upon it for a vital part of the raw materials from which their food and consequently their whole structure is elaborated.

The availability of the more common elements has been emphasized. The importance of their balance should also be noted. In this, lime should be included, for it is important not only in the maintenance of a desirable reaction and physical condition of the soil but calcium is among the more important elements necessary in the metabolism of plants.

Soil acidity is also an important consideration in many cane producing countries, but in Cuba the soils used for cane are, for the most part, slightly or actively alkaline. Elsewhere under conditions of excessive acidity, it has

been recognized for several years that the release of such toxic substances as aluminum, manganese, or iron has a deleterious effect upon plant growth; and, in relation to cane production in Hawaii and corn production in certain parts of the United States, this problem is receiving considerable study.

In the matter of definite soil type peculiarities that require attention in connection with general plant response, those associated with what has been tentatively classed as Oriente clay should be considered. In this the topsoil is a heavy black clay high in organic matter and usually low in lime. It cracks badly on drying and is underlain at 5 to 16 inches with a white chalky lime sometimes mixed with argillaceous material or in combination with hard or semi-hard limestone. It is a matter of common observation in many parts of the Island that this chalky material when brought into the zone of root development is very deleterious to plant growth. In fact, when the growing roots come in contact with this stratum they are said to turn quite upward and away from it. The appreciation of this effect is so general that in some instances deep plowing is assigned as the cause of crop failure in areas where this chalk could not possibly be reached with the plow. In such cases the degeneration of the cane was doubtless due rather to an impaired physical condition of the soil as a consequence of its improper handling. As noted above, cane is also commonly observed to do badly on many of the soils derived from serpentine. In some it will scarcely grow at all. Information at hand at the present time on the specific nature of the active principle involved in such instances is only sufficient for conjecture. Systematic studies in the laboratory and in the field upon this, as upon other broader relations, should yield information of the greatest importance, for analogous peculiarities in the interrelation of plant to soil type are to be found in other instances.

In the matter of simple physical injury to the living roots of the cane as a predisposing circumstance or condition to the entrance of the normal saprophytic fungi and bacteria of the soil into these structures, the situation is seriously complicated by certain soil-inhabiting insects that infest and feed upon these underground parts of the plants. Those that have been most commonly observed in this connection under field conditions in Cuba are the root mealybug, the white grub, termites, certain root borers, and, in very limited areas, wireworms. Any of these, through their natural feeding habits, open the plant to secondary parasites that commonly do much more serious damage than the original wounds inflicted by the insects themselves. In studying a definite situation involving failing or dying cane which has the appearance of root rot, therefore, care should be taken early in the examination to ascertain whether attacks of this nature may not represent the primary source of injury, due consideration of course being given to the possible cyclic or seasonal nature of their appearance in each instance.

Furthermore, important correlations are found to exist between soil type and the facility with which the infestation by some of these insects proceeds.

In general the relation is one of pulverulence or natural mulching (polvillo) upon drying. This point will be touched upon in some detail in a later paper. In this connection it is of interest to note the important part which snails are reported to be playing in this same rôle upon the roots of the cane in Louisiana.³ No significant infestations of this type have been observed by the writers in Cuba.

In regard to the irregular condition of the cane that is frequently found along the margins of the fields adjacent to the guardarrayas (firelanes) and particularly at the corners, it has been observed that this condition of the external growth is considered altogether too frequently as an indication that the entire field is suffering from root rot. It is found, however, that the more common cause for this condition is the promiscuous turning into the fields of the heavy cane carts, particularly during rainy weather; the foraging of animals, either while at work or at large; and also the simultaneous or subsequent encroachment of grass and weeds from the guardarraya. Where such parts of the field have been passed over repeatedly by carts and the soil thoroughly packed and in poor physical condition generally, considerable difficulty is frequently experienced in re-establishing the stand of cane. As noted above, this irregular condition of cane brought about either through physical injury from carts or bulls or through competition of weeds, or both, has been frequently referred to as root rot.

SUMMARY

The terms "root disease" or "root rot" have been applied to the dying of sugar cane under a variety of conditions, which are grouped as pathogenic, due to fungi and other parasitic organisms, and as non-pathogenic, due to lack of drainage, lack of moisture, deficiencies in fertility, cultivation, and other factors.

Of the various fungi isolated from diseased cane stools, two species of *Melanconium* were suspected of having a causal relation to the injury, but inoculations of cane plants in pots of sterilized soil with pure cultures and with decaying rootstocks gave negative results.

The field studies showed root disease to be associated with lack of aeration in undrained soils, with high salt content of the soil, with drought and resultant cracking of the soil, with high cutting and surface application of fertilizers, with infertile soils, and with the attacks on the roots of several insects and other small animals.

Improved agricultural practices are indicated as the most important means of relief.

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³ RANDS, R. D. Root disease of sugar cane in Louisiana. U. S. Dept. Agr. Circ. 366. 1926.

ZONATE FOOT ROT OF SUGAR CANE¹

JAMES A. FARIS

During the process of investigations to determine the primary causes for dying out of sugar cane plants (*Saccharum officinarum*) in the sugar estates in Cuba, a foot or basal stalk rot has been found which does not seem to have been previously recorded or described. This disease was first observed by the writer in parts of Oriente province in November, 1924, in spring planted cane of about eight months growth. During 1925 the disease was again found in the western part of Camagüey province upon stubble cane of the third ratoon. In each case the disease was quite



FIG. 1. Stool of sugar cane affected with zonate foot rot. Note the size of the stool and the number of shoots, many of which are dead.

¹ Scientific Contributions No. 5, Tropical Plant Research Foundation. from the Cuba Sugar Club Experiment Station, Central Baraguá, Cuba.

widely distributed over considerable areas within the immediate localities.

In the pathological collections of the Cuban Experiment Station at Santiago de las Vegas, a portion of a stalk of cane infected with this disease is reported² as having been sent to the station by a mill manager in Oriente province September 28, 1923. The present known distribution of the disease is therefore confined to widely separated localities in the provinces of Camagüey and Oriente, Cuba.

DESCRIPTION AND GENERAL CHARACTERS OF THE DISEASE

In both cases where this disease has been observed in the field, the dying plants were surrounded by very strong, vigorously growing cane. In the first instance a few hills along the edges of the fields were observed to be wilting, and a close examination of these stools revealed a large number of dead shoots (Fig. 1). After the trash had been cleared away it was found that every stalk in the stool was diseased, and that some of

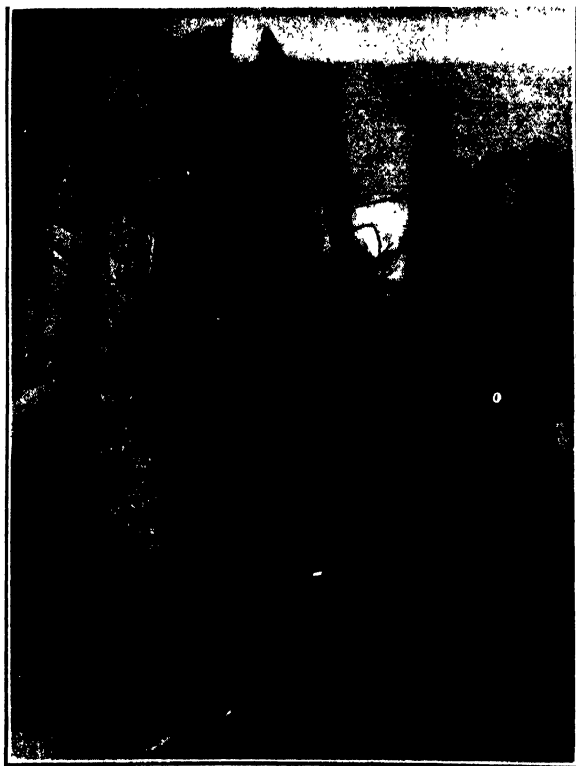


Fig. 2. Portion of a hill of cane affected with zonate foot rot.

² Personal letter from Stephen C. Bruner, Pathologist, Estacion Experimental Agronomica, to the author.

the larger stalks seemed to be dying rather suddenly. Such infected plants may occur singly or in spots of several hills. In one case an area was observed where six hills were dead. In another case two healthy plants were found entirely surrounded by plants dying of this zonate rot. In the primavera or spring cane the diseased plants were inconspicuous at first, as they were about the same height as the healthy cane. Whenever the foot rot had progressed far enough in a considerable number of stalks to stop the water supply, the hills presented the noticeably uneven, ragged appearance shown in figure 1.

Figure 2 is a photograph of a portion of a stool of cane which was killed by the zonate foot rot disease. This hill of cane produced 19 cane stalks which reached a good size before succumbing to the disease. Note the size of the stalks, many of which were $1\frac{1}{2}$ to 2 inches in diameter. No cane suitable for milling was harvested from this stool. The pithy hollow character noticeable at the tops of the stalks is often found in plants affected with this disease. This I attribute to the continued drain upon the moisture of the stalk after the root system has ceased functioning. Thus the stalk is dried out and becomes pithy and hollow between the nodes preceding decay from secondary fungi or bacteria. In some cases there is a central cylinder of brownish tissue whose color seems to be developed through chemical changes and degeneration of the cell walls of the center of the stalk. Surface sterilized plugs have been taken from this brown tissue above the area showing the zonated dry rot and plated in cane juice agar, but no organisms have developed.



FIG. 3. Surface view of a portion of a cane stalk affected with zonate foot rot. This shows the clear surface markings.

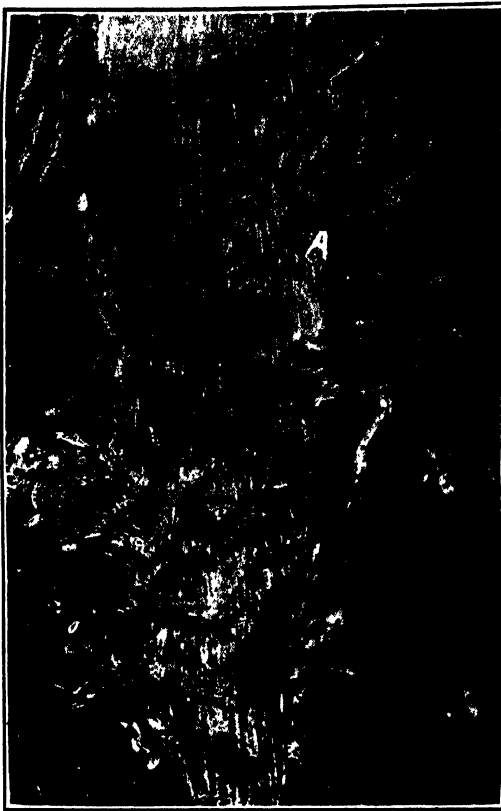


FIG. 4. Longitudinal section of the base of a cane stalk showing the characteristic zonate foot rot. At A is a root in which the cortex has been decayed.

SOIL TYPES CONCERNED

The disease has been found in considerable quantities upon two very different types of soil, one the Saltanejo or "hog wallow" soil of Oriente province, and the other a dark chocolate red soil of the Matanzas clay type. The former of these is very heavy black soil with a clay subsoil, and the latter is a porous red soil. Since these types are very different in their physical characteristics, soil solution, etc., there is little indication that the disease is limited to any great extent by soil type.

APPEARANCE OF DISEASED STALKS

At the bases of invaded stalks we find a zonate dry rot which leaves distinct rings of a blackish brown color upon the rind at that point. These ring-like zones are usually concave in form and more conspicuous above each node in the base of the diseased stalk. They are often obscure

until the wax coat of the stalk is removed, when they are very striking in appearance. Such zones are characteristic of no other known cane disease and serve as an easy method of separating this from other similar cane troubles.

Upon cutting either a cross or a longitudinal section of the stem it will be seen that these conspicuous zones on the rind are but the continuation of characteristic zones in the pith of the stem. Figure 3 shows these zones on the outer part of the rind. They tend to be concentric, but wider apart at the top the farther they are from the node.

A longitudinal section of the base of the stalk shows well defined alternating regions of gray and reddish tissues. The reddish bands are usually narrower than the gray, but both zones vary considerably in width. These zonated areas are below ground or within the first few inches above the soil surface. The cane cutters cut such stalks above the dry, woody portion; hence canes from the diseased areas might not indicate the presence of the infection. Figure 4 is a photograph of a longitudinal section through the base of a cane stalk with the characteristic zoning of the tissues. The alternating gray and reddish layers are more striking in appearance in fresh material than in the photographs. They are also more conspicuous in infected stalks which are still alive than in older dried stalks, as in the latter case the reddish zones may have almost faded out. In such cases the color usually remains in the more fibrous parts of the stem. The marks most persistent on the dead canes are the zones formed on the rind of the stalk.

The ring bordering upon the healthy tissues of the stem is a deep blood red in color. This is separated from the ring below by a somewhat wider grayish zone, and this in turn is followed by a brownish red zone. These zones fade to a brown or buff color when the tissues are completely dead. Above the last root ring shown in figure 4 the stalk was sound and showed no signs of rotting. However, the plant was wilting from the lack of moisture. It had a sufficiently well developed root system and plenty of rain had fallen to supply moisture, but the roots could not function because of the obstruction to the upward passage of the water by this dry rot.

Figure 5 shows the base of a stalk with an excellently developed root system, the lower part of which had lost its power to function because the woody zonated tissue prevented free communication with the stalk. This plant has tried to overcome the decreased supply of water by pushing out roots from the higher nodes. The disease has progressed to the node indicated by the arrow. Above this point the tissues of the stalk have every appearance of normal tissue. This sharp differentiation between the diseased and the healthy tissues by the advancing zone is characteristic of the rot.



FIG. 5. Longitudinal section of a sugar cane stalk affected with zonate foot rot. The arrow indicates the most advanced zone of infection.

A cross section of infected stalks shows the zonated condition to a very marked degree. Here the zones roughly approach concentric circles in form. The zone bordering the healthy tissue is a deep red color, and there is a marked tendency of the color to fade as the tissue rots. The infected tissue is dry and woody and thus in marked contrast to the fresh sappy pith of the portion of the stem not yet affected. Figure 6 is a photograph of sections of four infected stalks.

ENTRANCE INTO AND SPREAD THROUGH THE STALKS

The chief point of entrance of the fungus into the cane seems to be the base of the stalk. Some cases have been observed which suggested the possibility that the disease might begin at an infected root, but in every case the zones could be connected with others proceeding from the base of the stalk. Figure 4, A shows concentric rings formed around a root

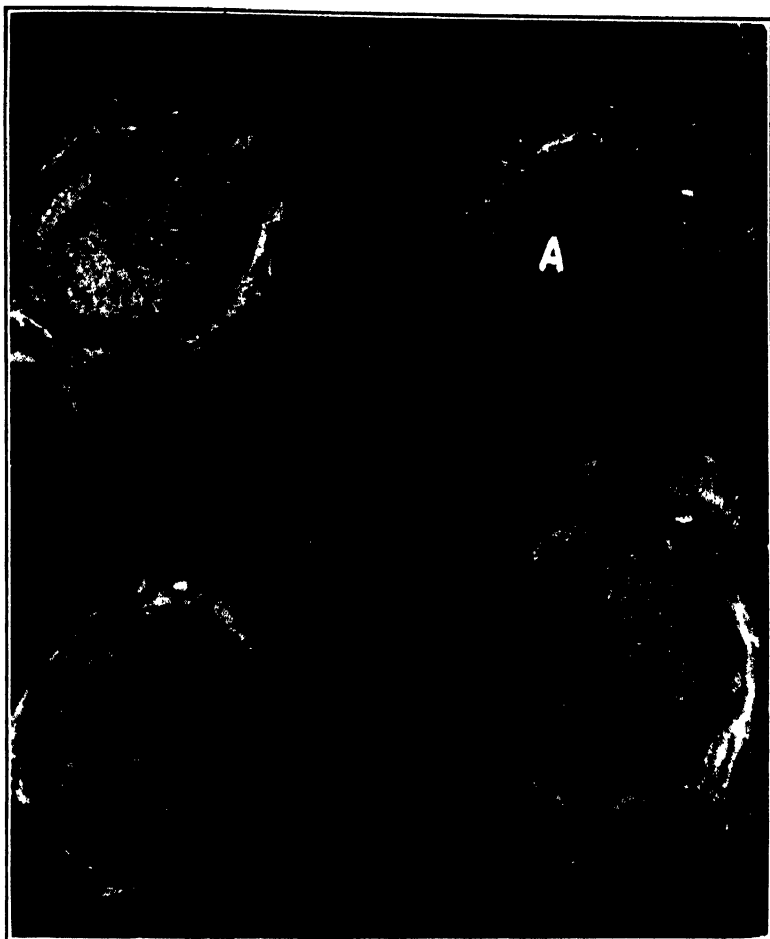


FIG. 6. Photograph of the cross section of four stalks of sugar cane infected with zonate foot rot. A shows a part of the stalk infected and a part uninfected. Note the sharp dividing line between the diseased and healthy pith.

entrance into the stalk. The cortex of the root was decayed but the central cylinder appeared to be little affected.

The disease has been traced in its course from the main primary stalk through the point of attachment into the secondary buds, from these through the point of attachment into the tertiary buds and into the shoots of the fourth rank. In every case the destruction was complete: the entire cepa or stool was dead. The zonate markings stay intact and continue through the woody underground parts of the stalks as well as through the internodes. Figure 8 is a diagrammatic longitudinal section showing the relation of the successive branches of the underground stems and the path of spread of the disease.

The infection starts from the infected ratoon or stubble cane as shown in figure 8, 1, and proceeds in turn into stalks number 2, 3, and 4. In many cases observed, the disease had spread to every shoot, both young and old, and the entire stools eventually died. In other instances a stool was found partially infected, but every indication pointed to complete destruction of the hill in time.

COMPARISON WITH SIMILAR ROOT AND STALK ROTS OF SUGAR CANE

In the root rot of sugar cane we have an infection confined to the roots of the plants in the early stages of the disease. Even plants badly stunted and with very short, stubby roots of one or two centimeters in length often show no signs of stalk rot, though the leaves may be wilting from lack of moisture. In the zonate foot rot the roots of the plant grow vigorously until the upward conduction of the sap is prevented by the dry



FIG. 7. Photograph of young shoots showing progress of zonate rot from older to younger tillers.

rot of the stalk base. In some cases the cortex of a root appears to be infected (Fig. 4, A), but in such cases it has not been determined whether the disease appeared first in the stalk and spread to the root or in the root first. From figure 4 it would seem that the disease had spread from the stalk to the root.

Both root rot and zonate foot rot usually appear in localized spots in the fields, but while root rot is largely confined to areas in which there is some conspicuous cultural defect, such as lack of drainage, etc., the zonate rot appears in vigorously growing cane under good cultural conditions.

In Cuba, as well as in other cane-growing countries, a root and basal stalk rot occurs which follows injury to cane weakened by other agencies, as, for example, high concentrations of injurious salts, soil deficiencies, drought, water-logged soils, and attacks of white grubs, root mealybugs, and wireworms. This is the type most in evidence in the Cuban cane fields. The final rotting is done largely by *Melanconium sacchari*, in some cases *M. ilia*, and some other fungi which have not fruited as yet in cultures. This is the type of trouble generally referred to locally as root disease. A number of experiments have been carried out with the above mentioned fungi, and with finely chopped diseased roots and stalk bases to determine whether these agencies are primarily responsible for the incidence of the disease. Experiments were carried out in both sterilized

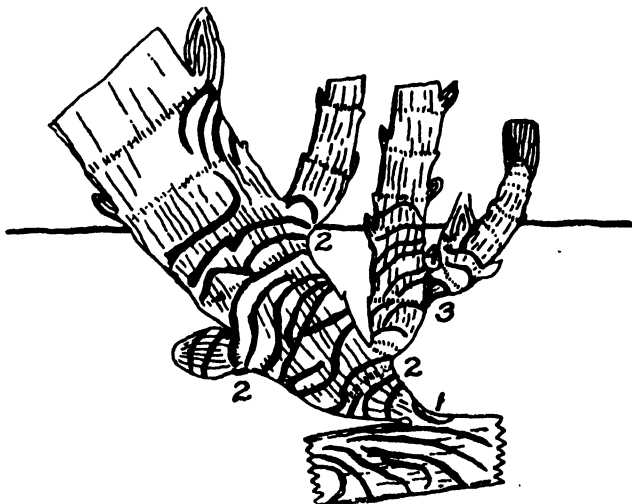


FIG. 8. Diagrammatic cross section of a portion of a stool of sugar cane showing the progress of the zonate foot rot. The disease enters the primary shoot (1) from the old infected stubble and progresses through shoots of 2nd, 3rd and 4th rank.

and unsterilized soil taken from diseased areas. No primary infection was secured. It is quite evident that previous weakening is necessary before cane plants succumb to this type of disease. The organisms seem to be semi-parasitic and play a secondary role in the injury of the plant.

In such diseased plants there are cylinders of infected bundle fibers extending through several internodes of the stalks. The plants attacked are stunted in their growth, have no marked zones at the base, but begin wilting and drying long before they finally die. The rot is not dry or woody, as it is in the case of the zonate rot.

Upon seeing cane affected with this zonate rot, Dr. G. Wilbrink, of Java, remarked to the writer that a similar disease is called Stengelbrand,

or stalk burning, in that country. This Javan disease is usually confined to sporadic cases in the young cane, but may become epidemic in a field. In affected plants the young spots on the stalks are a watery red color. The cells in these areas are all dead, the parenchyma dying first and later the sclerenchyma of the vascular strands. A marked difference in the Stengelbrand of Java and the zonate foot rot is the absence of zonated markings in the former. Also there are brown leaf spots associated with the Javan disease by which the affected plants may always be identified. No such spots have been observed in connection with the zonated rot.

A POSSIBLE CAUSE OF THE DISEASE

While making a periodic inspection in some fields in which there was zonate foot rot in the cane, plants were found with brackets of a *Fomes*



FIG. 9. Cane stalk affected with zonate foot rot, with the bracket of a species of *Fomes* attached.

species at the base. Longitudinal sections of these stalks showed the characteristic zoning at the ground surface and the fungous strands throughout the diseased portions of the stalks. The brackets were firmly attached to the stalks and so intimately associated with the zonated areas as strongly to suggest that this fungus was the probable causative organism. Figure 9 is a photograph of a portion of an affected stalk upon which has developed a bracket of this *Fomes*.

Specimens of such canes were submitted to Dr. J. R. Weir for identification, but the fungus was sterile. Dr. Weir states "the fungus is a species of *Fomes* with structure peculiar to a group typically represented by *F. pachyphloeus* Pat. and *F. melanodermus* Pat."

Figure 10 shows the brown fungous strands (A) throughout the stalk and the intimate association of the *Fomes* with the zonated portion of the cane stalk (B).

Many canes have been found which show only the zonate foot rot but no case has been found of a cane attacked by the *Fomes* which did not also show the zonated condition.



FIG. 10. Longitudinal section of two cane stalks affected with zonate foot rot upon which the *Fomes* species has developed. A. Brown strands of the *Fomes* throughout the cane stalk. B. *Fomes* intimately associated with the zonated parts of the stalk.

It seems quite possible that species of *Fomes* attacks some tree species in the forests, but neither its exact identification nor its host relationships have been determined. As pointed out before, the zonate foot rot of cane has been found only in fields where the cane had been planted in "monte" land, that is, immediately after the cutting of the forests.

SUMMARY

1. A description is given of a zonate foot rot of sugar cane which occurs in two widely separated localities in Cuba.
2. There is no indication that the disease is limited to certain types of soil, for it has been found on a very heavy black soil with a clay subsoil and on a porous red soil.
3. The disease is characterized by a zonate dry rot at the base of the stalk. Alternating bands of gray and reddish tissue are conspicuous above each node.
4. Fruiting bodies of a species of *Fomes* are often associated with the zonate rot. The species has not been identified, nor its host relationships determined.

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NATURE OF RESISTANCE OF BERBERIS SPP. TO PUCCINIA GRAMINIS¹

LEONARD W. MELANDER² AND J. H. CRAIGIE³

INTRODUCTION

It has been shown that the resistance of wheat varieties to the uredinal stage of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. may be due either to physiological or morphological causes. Furthermore, it has been shown that seedling plants may be susceptible to certain physiologic forms of the rust organism, whereas older plants of the same variety may be resistant (10). It also is known that the sporidial germ tubes of *P. graminis* mechanically force their way directly through the epidermis of the aecial host, and that young leaves of *Berberis vulgaris* L. are susceptible, while the older ones are resistant. This suggests the possibility that resistance may be due to the inability of the fungus to penetrate the epidermis of older leaves. Not only that, but it often has been observed that tough-leaved species of *Berberis* and *Odostemon* seem to be more resistant than tender-leaved species. It seemed possible, therefore, that the resistance of varieties of *Berberis* and *Odostemon* might be due to morphological differences. Therefore an investigation was undertaken to ascertain the following: (a) Can germ tubes of sporidia penetrate the cuticle of resistant varieties? (b) Is there a definite correlation between resistance to puncture and resistance to rust? (c) Is there a correlation between resistance to puncture and thickness of the cuticle or of the epidermal walls?

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HISTORICAL SUMMARY

It is well known that many plant pathogenes enter host plants by direct mechanical penetration of the epidermis. Brown (2), in 1916, found that the germ tubes of *Botrytis cinerea* Pers. were unable to affect chemically the cuticle of the host. Blackman and Welsford (1), in the same year, found that the piercing of the cuticle of *Vicia faba* by the germ tube of *Botrytis cinerea* Pers. was “. . . due solely to mechanical pressure exerted by the germ tube as a whole or by the special outgrowth from it.” In 1919 Dey (4) found that *Colletotrichum lindemuthianum* (Sacc. and Mag.) Bri. and Cav. formed thick-walled dark-colored appressoria, which became firmly attached to the host plant by a mucilaginous envelope. A peg-like infection hypha grew out from the appressorium and penetrated the cuticular layer by mechanical pressure. Leach (8) observed a similar phenomenon and showed that it took the hyphae longer to penetrate the cell walls of resistant than of susceptible varieties.

It has been known for some time that the germ tubes from sporidia of *Puccinia graminis* Pers. penetrate the cuticle of *Berberis* spp. directly, regardless of natural openings. Waterhouse (13) demonstrated that the penetration is accomplished by mechanical pressure and not by the dissolving action of enzymes.

In 1892 Cobb (3) suggested that resistance of certain Australian wheats to stem rust was correlated with the thickness and tensile strength of cuticle. Later Ward (12) concluded that morphological peculiarities had little effect on the resistance of a host. However, Hawkins and Harvey (5) showed that the resistance of the McCormick variety of potato to infection by *Pythium debaryanum* Hesse was correlated with resistance to mechanical puncture; and Hawkins and Sando (6) showed that cooling the fruit of strawberries, blackberries, black and red raspberries, and cherries made the epidermis more resistant to mechanical puncture. Valteau (11) found that varieties of plums having a thick, tough skin were more resistant to brown rot than thinner-skinned varieties. Willaman *et al* (14) also concluded that the toughness of the skin and the firmness of the flesh of plums are factors in resistance to brown rot. According to Hursh (7), varieties of wheat characterized by abundant sclerenchyma are likely to be injured less by stem rust because this tissue mechanically limits the spread of mycelium within the host. Melhus *et al* (9) suggested that the marked difference in the susceptibility of young and old leaves of the barberry might be due to the thickness of cuticle and epidermis, considering that infection is accomplished by direct penetration of the tissues and not through invasion of stomata.

MATERIALS AND METHODS

To determine whether the mycelium of *Puccinia graminis* actually enters immune plants, leaves of *Berberis thunbergii* D. C. were inoculated and incubated 4, 5, 6, 7, and 13 days. They then were killed, embedded in paraffin, sectioned, and stained with Fleming's triple stain. They next were examined to ascertain whether the rust mycelium had entered. Observations also were made on fresh leaves of *B. thunbergii* which had been inoculated with sporidia of *P. graminis* Pers.

For determining the thickness of the external walls of the epidermal cells, fresh leaves were taken from plants of various species of *Berberis* growing in the greenhouse. Hand-sections of these leaves were made and measurements taken by means of a filar micrometer. In several cases, fresh leaves of the desired age were not available, so the measurements were made on leaves previously mounted in paraffin. All measurements were made at approximately the middle of the cell wall, thus avoiding the thickenings at the corners of the cells. Leaves of three ages were chosen: 2 to 3 days; 5 to 6 days; and 16 to 20 days, designated in table 1 as mature. Measurements for the two-day-old and three-day-old leaves were tabulated separately at first; but, as they were almost identical, there was no necessity of keeping them separate. Readings for leaves 5 and 6 days old were grouped together for the same reason.

Whenever practicable, readings for any particular age were taken from leaves of several plants of the same species. This could not always be done, as in the case of *B. pruinosa* and *B. lycium*, in which the readings had to be made from leaves of one plant. In general, 20 readings were taken for each leaf of a given age and the averages were based on 100 readings. In some cases, many more readings were made; but the averages, as given in table 1, were not affected thereby. Measurements also were made of the cell walls on the lower surface of the leaves, but, as they usually corresponded very closely with those on the upper side of the leaves, it is not necessary to consider them further.

The thickness of the cuticle as such was not measured. It was so thin in very young leaves, if present at all, that it could not be measured. In the older leaves it was included as part of the cell wall.

Ten-day-old leaves of *B. vulgaris* were sectioned in order to compare the thickness of the cell walls with that of the younger leaves of other species.

A modified Jolly balance (Fig. 4), fitted with a specially ground, round-pointed phonograph needle, was used to measure the pressure required to puncture the cuticle. The approximate diameter of this needle was only 25 microns, and a binocular magnifier was required to see when it had pene-

trated the cuticle. One centimeter on the vernier was equal to a pressure of 518 milligrams. To make conditions as uniform as possible, the puncturing always was done early in the afternoon of sunny days. To insure uniform leaf turgor, plants were maintained in moist soil. As a rule, only five measurements were possible for each leaf because the leaves soon wilted after being cut from the plant and the resistance of wilted leaves to puncture was different from that of turgid leaves. Twenty-five measurements were made of leaves of each species at each age. The ages of the leaves used were 1, 2, 3, 4, 7, 10, and 14 days. The following is a list of the species of *Berberis* and *Odostemon* which were studied:

Susceptible	Resistant	Immune
<i>B. aristata</i> D. C.	<i>B. brachypoda</i> Maxim.	<i>B. thunbergii</i> D. C.
<i>B. buxifolia</i> Lam.	<i>B. chinensis</i> Poiret	<i>O. repens</i> (Lindl.) Rydb.
<i>B. canadensis</i> Mill.	<i>B. lycium</i> Royle	
<i>B. dictyophylla</i> Franch	<i>B. pruinosa</i> Franch	
<i>B. leichlinii</i>	<i>O. aquifolium</i> (Pursh)	
<i>B. vulgaris</i> Linn.	Rydb.	
<i>O. swaseyi</i> (Buckl.) Rydb.		

RESULTS

A few preliminary attempts made to determine whether the sporidial germ tube of *Puccinia graminis* actually enters the immune *B. thunbergii* yielded no evidence that the parasite penetrates the outer epidermal wall. However, these studies were not extensive enough to justify final conclusions.

Thickness of outer walls of epidermal cells. Outer walls of the epidermal cells of susceptible varieties were found to be thinner than those of resistant or immune varieties, with the exception of *B. pruinosa* (Table 1). The epidermal cells of this species are quite small, scarcely more than one-third the width of the cells of many of the other species, and have very heavy perpendicular walls, much thickened at the angles. The result is that the central portion of the external wall of the epidermis (the portion for which the measurement is given in table 1) is very much thinner than either of the end portions. Consequently the mean thickness of the cell wall would be almost twice as great as the thickness given in table 1. The walls of two-to-three-day-old leaves of the immune *B. thunbergii* are almost twice as thick as those of the susceptible *B. dictyophylla*, and those of the immune *Odostemon repens* more than twice as thick as those of *B. dictyophylla* and *B. canadensis*, both of which are susceptible.

It will be noted (Table 1) that the thickness of the epidermal walls increases with age and that this increase is more rapid in resistant than in susceptible varieties. Therefore the period of susceptibility of

resistant varieties probably is shorter than that of susceptible varieties. The epidermal walls of the immune *O. repens* are very thick and are covered with a waxy exudate even when the leaves are very young.

TABLE 1.—Average thickness of the external walls of epidermal cells of the upper surface of leaves of various species of *Berberis*

Species	Age of leaves		
	2 and 3 days	5 and 6 days	Mature
	Thickness in microns		
Susceptible			
<i>Berberis canadensis</i>	0.88	0.93	1.29
<i>B. dictyophylla</i>	0.82	1.23	1.80
<i>B. vulgaris</i>	1.10	1.18	1.87
Resistant			
<i>B. brachypoda</i>	1.43	2.09	2.56
<i>B. lycium</i>	1.23	2.86	3.41
<i>B. pruinosa</i>	1.16	1.46	2.20
<i>Odostemon aquifolium</i>	1.30 ^a	3.19 ^a
Immune			
<i>B. thunbergii</i>	1.57	1.62	2.44
<i>O. repens</i>	1.75 ^a	3.01

^a Measurements from killed material mounted in paraffin.

But the leaves of susceptible species also become practically immune with age. The results obtained in these studies show that this may be due, at least partly, to the thickening of the epidermal cell wall. Ten-day-old leaves of *B. vulgaris* (not included in table 1) have an average cell wall thickness of 1.52 microns, which is slightly less than that of the two- and three-day-old leaves of *B. thunbergii*. Leaves of *B. vulgaris* ten days old are fairly resistant to *P. graminis*. This would indicate a close correlation between resistance and the thickness of the outer epidermal wall. The thickness of the wall, however, does not seem to be the only factor, as young leaves of *B. brachypoda* have comparatively thick-walled epidermal cells but become infected readily, forming numerous pycnia and but few aecia. Obviously the germ tubes have entered the leaves, but some condition inside the leaf prevents normal development.

Resistance to puncture. The data on the resistance to puncture of leaves of various species of *Berberis* and *Odostemon* are given in tables 2, 3, 4, 5, 6, 7, 8 and 9. If the resistance of various species of *Berberis* is due to the toughness of the epidermal walls, no species would be more resistant to the rust parasite than the most tender portion of the outer cell walls.

TABLE 2.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of one-day-old leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture										Constants			
	Class centers in milligrams										Range	Mean	Standard deviation	Coefficient of variability
	200	250	300	350	400	450	500	550	600					
Susceptible														
<i>B. aristata</i>	1	1	10	10	2	1				207.2—440.3	328 ± 6.62	49.0 ± 4.67	14.94 ± 1.43	
<i>B. buxifolia</i>		3	5	12	5					233.1—388.5	338 ± 6.11	45.3 ± 4.32	13.40 ± 1.28	
<i>B. canadensis</i>		2	8	11	4					259.0—388.5	334 ± 5.64	41.8 ± 3.99	12.51 ± 1.19	
<i>B. dictyophylla</i>		2	14	7	2					259.0—388.5	318 ± 5.00	37.1 ± 3.54	11.67 ± 1.11	
<i>B. leichlinii</i>		2	4	9	8	2				259.0—466.2	358 ± 7.06	52.3 ± 4.99	14.61 ± 1.39	
<i>B. swaseyi</i>		1	7	8	8	1				259.0—440.3	352 ± 6.46	47.9 ± 4.57	13.61 ± 1.30	
<i>B. vulgaris</i>		2	13	8	2					233.1—388.5	320 ± 5.05	37.4 ± 3.57	11.69 ± 1.12	
Resistant														
<i>B. brachypoda</i>		5	10	5	4	1				233.1—466.2	322 ± 7.42	55.0 ± 5.24	16.77 ± 1.60	
<i>B. chinensis</i>				9	8	7	1			336.7—492.1	400 ± 6.03	44.7 ± 4.26	11.18 ± 1.07	
<i>B. lycium</i>			3	9	9		2	2		310.8—569.8	390 ± 9.15	67.8 ± 6.47	17.38 ± 1.66	
<i>B. pruinosa</i>		1		8	10	5	1			290.0—492.4	392 ± 6.79	50.3 ± 4.80	12.83 ± 1.22	
<i>O. aquifolium</i>		1	5	15	4					259.0—414.4	344 ± 4.80	35.6 ± 3.40	10.35 ± 0.99	
Immune														
<i>B. thunbergii</i>						4	10	6	5	440.3—595.7	524 ± 6.64	49.2 ± 4.69	9.39 ± 0.90	
<i>O. repens</i>		3	10	6	6					233.1—414.4	330 ± 6.61	49.0 ± 4.67	14.85 ± 1.42	

TABLE 3.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of two-day-old leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture										Constants			
	Class centers in milligrams										Range	Mean	Standard deviation	Coefficient of variability
	200	250	300	350	400	450	500	550	600	650				
Susceptible														
<i>B. aristata</i>			1	9	8	7					310.8—466.2	392 ± 5.94	44.0 ± 4.20	11.22 ± 1.07
<i>B. buxifolia</i>				2	16	7					336.7—466.2	410 ± 3.82	28.3 ± 2.70	6.90 ± 0.66
<i>B. canadensis</i>		1	5	12	6	1					259.0—440.3	352 ± 5.87	43.5 ± 4.15	12.36 ± 1.18
<i>B. dictyophylla</i>		1	3	11	7	3					259.0—466.2	366 ± 6.20	46.0 ± 4.38	12.77 ± 1.21
<i>B. leichlinii</i>		1	7	8	6	2	1				259.0—492.1	358 ± 7.80	57.8 ± 5.51	16.15 ± 1.54
<i>B. vulgaris</i>			5	10	5	5					284.9—492.1	370 ± 6.88	51.0 ± 4.86	13.78 ± 1.31
<i>O. swaseyi</i>			4	8	8	5					310.8—466.2	378 ± 6.64	49.2 ± 4.69	13.02 ± 1.24
Resistant														
<i>B. brachypoda</i>			8	17							284.9—362.6	334 ± 3.14	23.3 ± 2.22	6.98 ± 0.67
<i>B. chinensis</i>					6	5	7	6	1		388.5—595.7	482 ± 8.02	59.5 ± 5.66	12.34 ± 1.18
<i>B. lycium</i>					6	11	6	2			388.5—569.8	458 ± 5.94	44.0 ± 4.20	9.61 ± 0.92
<i>B. pruinosa</i>				4	6	5	9	1			569.8—336.7	444 ± 7.95	58.9 ± 5.62	13.27 ± 1.27
<i>O. aquifolium</i>			2	6	12	3	2				310.8—518.0	394 ± 6.69	49.6 ± 4.73	12.59 ± 1.20
Immune														
<i>B. thunbergii</i>							1	6	11	6	1	600 ± 6.03	44.7 ± 4.26	7.45 ± 0.71
<i>O. revens</i>				9	10	5	1				336.7—492.1	396 ± 5.69	42.2 ± 4.03	10.66 ± 1.02

TABLE 4.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of three-day-old leaves of various species of *Berberis* and *Oostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture												Constants				
	Class centers in milligrams												Range	Mean	Standard deviation	Coefficient of variability	
	250	300	350	400	450	500	550	600	650	700	750	800					850
Susceptible																	
<i>B. aristata</i>		1	2	3	9	7	3							318.8—569.8	456 ± 8.39	62.2 ± 5.93	13.64 ± 1.30
<i>B. buxifolia</i>	2	4	2	8	9									233.1—466.2	386 ± 8.84	65.6 ± 6.26	16.99 ± 1.62
<i>B. canadensis</i>			2	7	9	7								362.6—492.1	442 ± 6.23	46.2 ± 4.41	10.45 ± 1.00
<i>B. dictyophylla</i>			2	2	5	6	4	3	3					362.6—673.4	508 ± 11.53	85.5 ± 8.15	16.83 ± 1.61
<i>B. leichlinii</i>				3	12	6	3	1						414.4—595.7	474 ± 6.64	49.2 ± 4.69	10.38 ± 0.99
<i>B. vulgaris</i>	1	3	6	3	5	6	1							259.0—543.9	410 ± 10.79	80.0 ± 7.63	19.51 ± 1.86
<i>O. swaseyi</i>		1	3	10	9	1	1							284.9—543.9	418 ± 6.85	50.8 ± 4.85	12.15 ± 1.16
Resistant																	
<i>B. brachypoda</i>		6	12	6	1									284.9—466.2	354 ± 5.37	39.8 ± 3.80	11.24 ± 1.07
<i>B. chinensis</i>		1	3	3	8	5	3	1	1					310.8—673.4	462 ± 10.85	80.4 ± 7.67	17.40 ± 1.66
<i>B. lycium</i>					2	7	6	4	2	2	2			466.2—751.1	572 ± 11.46	85.0 ± 8.11	14.86 ± 1.43
<i>O. aquifolium</i>				3	7	9	2	4						414.4—595.7	494 ± 8.16	60.5 ± 5.77	12.25 ± 1.17
Immune																	
<i>B. thumbergii</i>					3	12	6	2	2					440.3—647.5	526 ± 7.16	53.1 ± 5.07	10.10 ± 0.96
<i>O. repens</i>								5	5	6	6	2	1	595.7—854.7	696 ± 9.34	69.2 ± 6.60	9.94 ± 0.95

TABLE 5.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of four-day-old leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture											Constants		
	Class centers in milligrams											Range	Mean	Standard deviation
	350	400	450	500	550	600	650	700	750	800	850			
Susceptible														
<i>B. aristata</i>	1	9	6	2	5		2					362.6—647.5	468 ± 10.77	79.8 ± 7.61
<i>B. buxifolia</i>	1	5	13	6		1	1					362.6—518.0	448 ± 5.22	38.7 ± 3.69
<i>B. canadensis</i>		4	12	5	2	1						388.5—647.5	474 ± 8.12	60.2 ± 5.74
<i>B. decyophylla</i>					1	5	7	11	1			543.9—751.1	662 ± 6.41	47.5 ± 4.53
<i>B. leichlinii</i>			1	5	6	5	2	5	1			466.2—751.1	592 ± 10.91	80.9 ± 7.72
<i>B. vulgaris</i>	1	10	9	3	1	1						362.6—595.7	442 ± 7.31	54.2 ± 5.17
<i>O. swaseyi</i>			5	5	5	3	5	2				440.3—699.3	558 ± 10.91	80.9 ± 7.72
Resistant														
<i>B. brachypoda</i>	4	7	6	6	2							336.7—569.8	440 ± 8.09	60.0 ± 5.72
<i>B. chinensis</i>			1	2	6	5	10	1				466.2—699.3	598 ± 8.20	60.8 ± 5.80
<i>B. lycium</i>			3	3	6	11	2					440.3—673.4	562 ± 7.70	57.1 ± 5.45
<i>O. aquifolium</i>			1	4	11	2	3	4				466.2—725.2	578 ± 9.55	70.8 ± 6.75
Immune														
<i>B. thunbergii</i>				3	7	13	2					466.2—647.5	578 ± 5.42	40.2 ± 3.83
<i>O. repens</i>					3	1	3	7	3	5	3	569.8—854.7	716 ± 12.17	90.2 ± 8.61

TABLE 6.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of seven-day-old leaves of various species of *Berberis* and *Odoemeon*, as determined by a modified Jolly balance

Variety	Resistance to puncture										Constants			
	Class centers in milligrams										Range	Mean	Standard deviation	Coefficient of variability
	300	400	500	600	700	800	900	1000						
Susceptible														
<i>B. aristata</i>	1	13	8	3					414.4—	673.4	552 ± 10.18	75.5 ± 7.20	13.68 ± 1.30	
<i>B. buxifolia</i>	1	9	14	1					466.2—	699.3	640 ± 8.53	63.2 ± 6.03	9.88 ± 0.94	
<i>B. canadensis</i>	4	11		2	5	3			466.2—	1010.1	612 ± 23.31	172.8 ± 16.48	28.23 ± 2.69	
<i>B. dictyophylla</i>	3		6	2	5	4	5		362.6—	880.6	664 ± 23.18	171.8 ± 16.39	25.87 ± 2.47	
<i>B. leichlinii</i>			1	13	10		1		543.9—	854.7	648 ± 10.18	75.5 ± 7.20	11.65 ± 1.11	
<i>O. swaseyi</i>				3	11	6	5		492.1—	828.8	752 ± 12.72	94.3 ± 9.00	12.54 ± 1.26	
<i>B. vulgaris</i>	1	2	5	3	8	5	1		284.9—	880.6	636 ± 20.13	149.2 ± 14.23	23.45 ± 2.25	
Resistant														
<i>B. brachypoda</i>			4	11	6	4			466.2—	828.8	640 ± 12.65	93.8 ± 8.95	14.66 ± 1.40	
<i>B. chinensis</i>	7	18							362.6—	518.0	472 ± 6.06	44.9 ± 4.28	9.51 ± 0.91	
<i>B. lycium</i>				5	7	11	2		569.8—	880.6	740 ± 12.06	89.4 ± 8.53	12.08 ± 1.15	
<i>O. aquifolium</i>			3	11	9	1	1		518.0—	880.6	644 ± 12.11	89.8 ± 8.57	13.94 ± 1.33	
Immune														
<i>B. thunbergii</i>				12	9	4			569.8—	802.9	668 ± 9.89	73.3 ± 6.99	10.97 ± 1.05	
<i>O. repens</i>				2	8	9	5	1	647.5—	958.3	780 ± 13.22	98.0 ± 9.35	12.56 ± 1.20	

TABLE 7.—Variations in and constants of the resistance to puncture of the outer walls of epidermal cells of ten-day-old leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture										Constants				
	Class centers in milligrams										Range	Mean	Standard deviation	Coefficient of variability	
	400	500	600	700	800	900	1000	1100	1200	1300					
Susceptible															
<i>B. aristata</i>			1	5	12	4	1	2			647.5—1061.9	820 ± 15.73	116.6 ± 11.12	14.22 ± 1.36	
<i>B. buxifolia</i>	8	10		7							492.1—725.2	596 ± 10.44	77.4 ± 7.38	12.99 ± 1.24	
<i>B. canadensis</i>			7	4	6	4	2	2			621.6—1061.9	784 ± 21.14	156.7 ± 14.95	19.99 ± 1.91	
<i>B. leichlinii</i>	6	5	5	7	2	4	1				466.2—958.3	684 ± 20.07	148.8 ± 14.19	21.75 ± 2.07	
<i>B. vulgaris</i>	5	10	4	4	4	2					518.0—880.6	652 ± 16.24	120.4 ± 11.48	18.47 ± 1.76	
<i>O. swaseyi</i>				1	3	6	9	6			751.1—1139.6	964 ± 14.72	109.1 ± 10.41	11.32 ± 1.08	
Resistant															
<i>B. brachypoda</i>	2	9	6	7	1						518.0—854.7	684 ± 14.11	104.6 ± 9.98	15.29 ± 1.46	
<i>B. chinensis</i>	5	17	2	1							414.4—673.4	496 ± 8.93	66.2 ± 6.31	16.35 ± 1.27	
<i>B. lycium</i>			2	2	14	6	1				647.5—958.3	808 ± 12.02	89.1 ± 8.50	11.03 ± 1.05	
<i>O. aquifolium</i>	1	1	1	8	9	5	1				518.0—1010.1	776 ± 14.42	106.9 ± 10.20	13.78 ± 1.31	
Immune															
<i>B. thunbergii</i>				4	14	5	2				699.3—1036.0	820 ± 10.79	80.0 ± 7.63	9.76 ± 0.93	
<i>O.</i>					1	0	10	4	1		898.8—1165.5	980 ± 19.06	90.4 ± 9.52	0.19 ± 0.07	

TABLE 8.—Variations in \bar{u} and constants of the resistance to puncture of the outer walls of epidermal cells of fourteen-day-old leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Resistance to puncture																				
	Class centers in milligrams																				
	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	
Susceptible																					
<i>B. aristata</i>				1	6	7	9	2													
<i>B. buxifolia</i>					4	9	9	2	1												
<i>B. canadensis</i>					4	7	2	6	5	1											
<i>B. leichlinii</i>		1	1	1	1	5	9	5	2												
<i>B. vulgaris</i>		1	9	6	6	3															
<i>O. swaseyi</i>								2	5	4	5	1	3	1	1	1	1			1	
Resistant																					
<i>B. brachypoda</i>						10	4	8	3												
<i>B. chinensis</i>	4	11	7	3																	
<i>B. lycium</i>			1	4	6	6	7	1													
<i>O. aquifolium</i>				2	11	8	2	2													
Immune																					
<i>B. thunbergii</i>						10	12	3													
<i>O. repens</i>						1	4	10	5	3	1		1								

TABLE 8.—(Continued)

Variety	Constants			
	Range	Mean	Standard deviation	Coefficient of variability
Susceptible				
<i>B. aristata</i>	751.1-1061.9	920 ± 13.75	101.9 ± 9.72	11.08 ± 1.06
<i>B. buxifolia</i>	751.1-1191.4	948 ± 13.29	98.5 ± 9.40	10.39 ± 0.99
<i>B. canadensis</i>	828.8-1372.7	1020 ± 21.58	160.0 ± 15.26	15.69 ± 1.50
<i>B. leichlinii</i>	518.0-1243.2	960 ± 22.25	164.9 ± 15.73	17.18 ± 1.64
<i>B. vulgaris</i>	466.2- 880.6	704 ± 15.01	111.3 ± 10.62	15.81 ± 1.51
<i>O. swaseyi</i>	1061.9-2305.1	1456 ± 39.49	292.7 ± 27.92	20.10 ± 1.92
Resistant				
<i>B. brachypoda</i>	854.7-1243.2	1016 ± 14.62	108.4 ± 10.34	10.67 ± 1.02
<i>B. chinensis</i>	362.6- 673.4	536 ± 11.99	88.9 ± 8.48	16.59 ± 1.58
<i>B. lycium</i>	647.5-1061.9	868 ± 16.94	125.6 ± 11.98	14.47 ± 1.38
<i>O. aquifolium</i>	725.2-1061.9	864 ± 13.69	101.5 ± 9.68	11.75 ± 1.12
Immune				
<i>B. thumbergii</i>	880.6-1061.9	972 ± 8.96	66.4 ± 6.33	6.83 ± 0.65
<i>O. repens</i>	932.4-1605.8	1152 ± 19.51	144.6 ± 13.79	12.55 ± 1.20

TABLE 9.—Summary of the mean pressures in milligrams required to penetrate the outer walls of epidermal cells of leaves of various species of *Berberis* and *Odostemon*, as determined by a modified Jolly balance

Variety	Mean pressure in milligrams							
	Age of leaves in days							
	1	2	3	4	7	10	14	
Susceptible								
<i>B. aristata</i>	328 ± 6.62	392 ± 5.94	456 ± 8.39	468 ± 10.77	552 ± 10.18	820 ± 15.73	920 ± 13.75	
<i>B. buxifolia</i>	338 ± 6.11	410 ± 3.82	386 ± 8.84	448 ± 5.22	640 ± 8.53	596 ± 10.44	948 ± 13.29	
<i>B. canadensis</i>	334 ± 5.64	352 ± 5.87	442 ± 6.23	474 ± 8.12	612 ± 23.31	784 ± 21.14	1020 ± 21.58	
<i>B. dictyophylla</i>	318 ± 5.00	366 ± 6.20	508 ± 11.54	662 ± 6.41	644 ± 23.18	
<i>B. leichlinii</i>	358 ± 7.06	358 ± 7.80	474 ± 6.64	592 ± 10.91	648 ± 10.18	684 ± 20.07	960 ± 25.25	
<i>O. swayseyi</i>	352 ± 6.46	378 ± 6.64	418 ± 6.85	558 ± 9.85	752 ± 12.72	964 ± 14.72	1456 ± 39.49	
<i>B. vulgaris</i>	320 ± 5.05	370 ± 6.88	410 ± 10.79	442 ± 10.91	636 ± 20.13	652 ± 16.24	704 ± 15.01	
Resistant								
<i>B. brachypoda</i>	322 ± 7.42	334 ± 3.14	354 ± 5.37	440 ± 8.09	640 ± 12.65	684 ± 14.11	1016 ± 14.62	
<i>B. chinensis</i>	400 ± 6.03	482 ± 8.02	462 ± 10.85	598 ± 8.20	472 ± 6.06	496 ± 8.93	536 ± 11.99	
<i>B. lycium</i>	390 ± 9.15	458 ± 5.94	572 ± 11.46	562 ± 7.70	740 ± 12.06	808 ± 12.02	868 ± 16.94	
<i>B. pruinosa</i>	392 ± 6.79	444 ± 7.95	
<i>O. aquifolium</i>	344 ± 4.80	394 ± 6.69	494 ± 8.16	578 ± 9.55	644 ± 12.11	776 ± 14.42	864 ± 13.69	
Immune								
<i>B. thunbergii</i>	524 ± 6.64	600 ± 6.03	526 ± 7.16	578 ± 5.42	668 ± 9.89	820 ± 10.79	972 ± 8.96	
<i>O. repens</i>	330 ± 6.61	396 ± 5.69	696 ± 9.34	716 ± 12.17	780 ± 13.22	980 ± 12.06	1152 ± 19.51	

Young leaves are most easily punctured and are also most susceptible to stem rust. In general, one-day-old leaves of susceptible varieties were found to be less resistant to puncture than those of resistant or immune varieties. The difference between the resistance to puncture of one-day-old leaves of *B. thunbergii* (immune) and *B. vulgaris* (susceptible) is more than 2½ times the probable error of the difference. It will be noted that one-day-old leaves of *B. chinensis*, which is definitely known to be quite resistant, has a puncture index intermediate between those of the susceptible and immune varieties. The somewhat resistant *B. brachypoda*, with a puncture index equal to that of susceptible species, may possess some internal resistance. One-day-old leaves of *B. pruinosa* (resistant) have a high mean index of resistance to puncture, but it will be noted that it also has a small minimum and a large maximum (Table 2). The nature of the epidermal cells of this species already has been noted. Owing to the fact that the epidermal cells are small, there are more of the thick perpendicular walls per unit of area to lend support to the horizontal walls when pressure

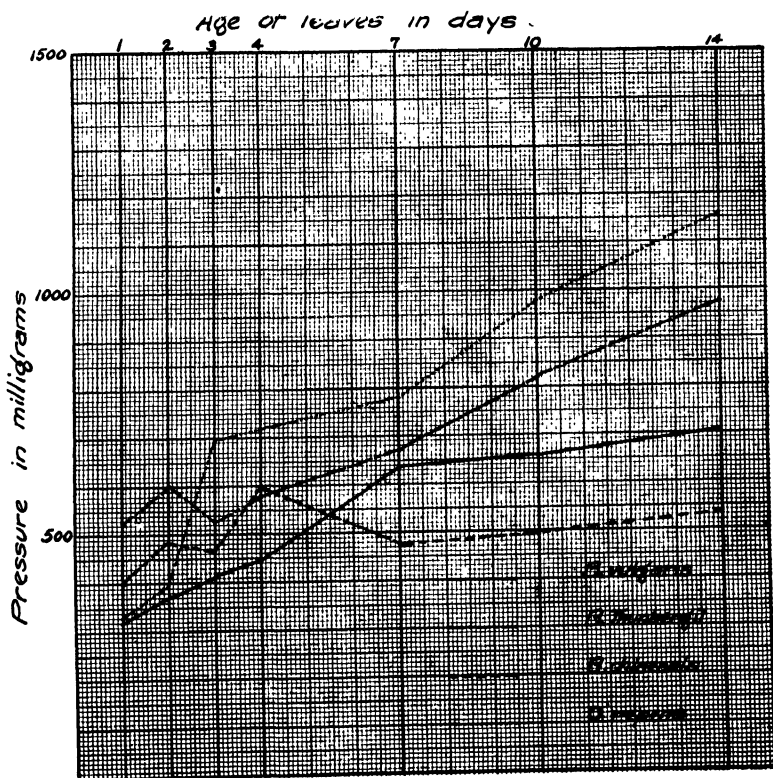


FIG. 1. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. vulgaris*, *B. thunbergii*, *B. chinensis*, and *O. repens*, as measured by a modified Jolly balance.

is exerted upon them, and the thick perpendicular walls possibly may be a factor in preventing the growth of hyphae after entrance has been gained.

It will be noted also that the puncture index for one-day-old leaves of *Odostemon repens* is rather low. It already has been pointed out that the epidermal walls of the young leaves of this species are quite thick. The low resistance of one-day-old leaves to puncture might be explained by the fact that the cuticle of the leaves of this age is merely a waxy exudate which would offer very little resistance to puncture. In this case the pressure required to puncture the epidermal wall would be practically the resistance of the epidermal wall without the cuticle. Starting with the second day, however, the cuticle hardens rapidly and becomes very resistant to puncture (Fig. 1).

Reference already has been made to the rapidity with which the resistance to puncture of some species of *Berberis* and *Odostemon* increases. The resistance to puncture increases at about the same rate as the thickening of the epidermal wall and cuticle, although toughness and not mere

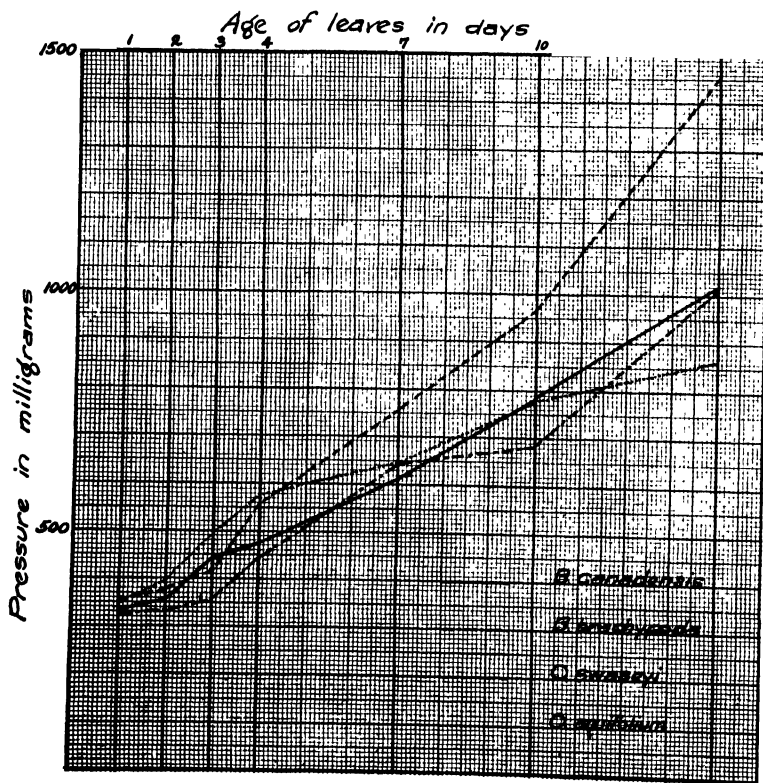


FIG. 2. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. canadensis*, *B. brachypoda*, *O. swaseyi*, and *O. aquifolium*, as measured by a modified Jolly balance.

thickness may also be very important. While the puncture index of one-day-old leaves of *O. repens* is approximately the same as that of leaves of *B. vulgaris* of the same age, the index of three-day-old leaves is 50 per cent greater than that of leaves of *B. vulgaris* of the same age. In fact, the three-day-old leaves of *O. repens* are more resistant to puncture than those of *B. thunbergii*. This would mean that the soft cuticle must have hardened and resistance may be due partly to the rapid hardening of the cuticle. Leaves of *B. vulgaris* increase in toughness with age: seven-day-old leaves are more resistant to puncture than one-day-old leaves of *B. thunbergii*. While the one-day-old leaves of *B. chinensis* are intermediate in their resistance to puncture, they do not seem to increase in toughness like those of many of the other varieties. The tendency of most species to toughen quite rapidly probably explains the tendency of the leaves of susceptible species to become practically immune with age (Figures 1, 2, and 3).

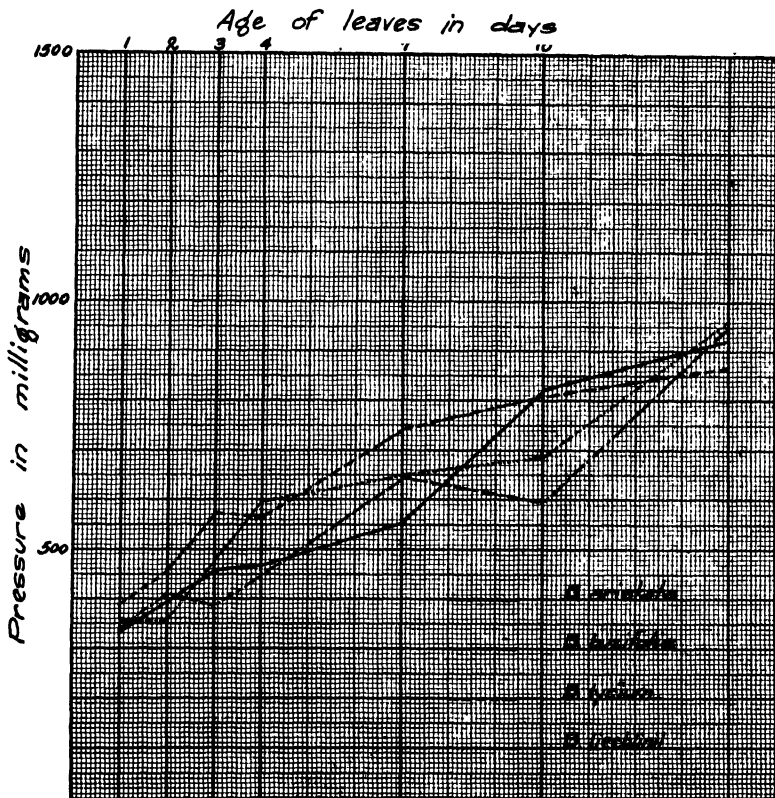


FIG. 3. Variation in resistance to puncture of the outer epidermal wall of leaves of *B. aristata*, *B. buxifolia*, *B. lycium*, and *B. sieboldii*, as measured by a modified Jolly balance.

CONCLUSIONS

The results of the investigation show rather conclusively that barberries may be resistant to *Puccinia graminis* because of morphological characters. It is obvious, however, that this does not necessarily explain the resistance of all varieties. There evidently is also a physiological resistance. The germ tubes are unable to penetrate the epidermal cell walls of some varieties, but can penetrate those of other varieties readily. Subsequent devel-

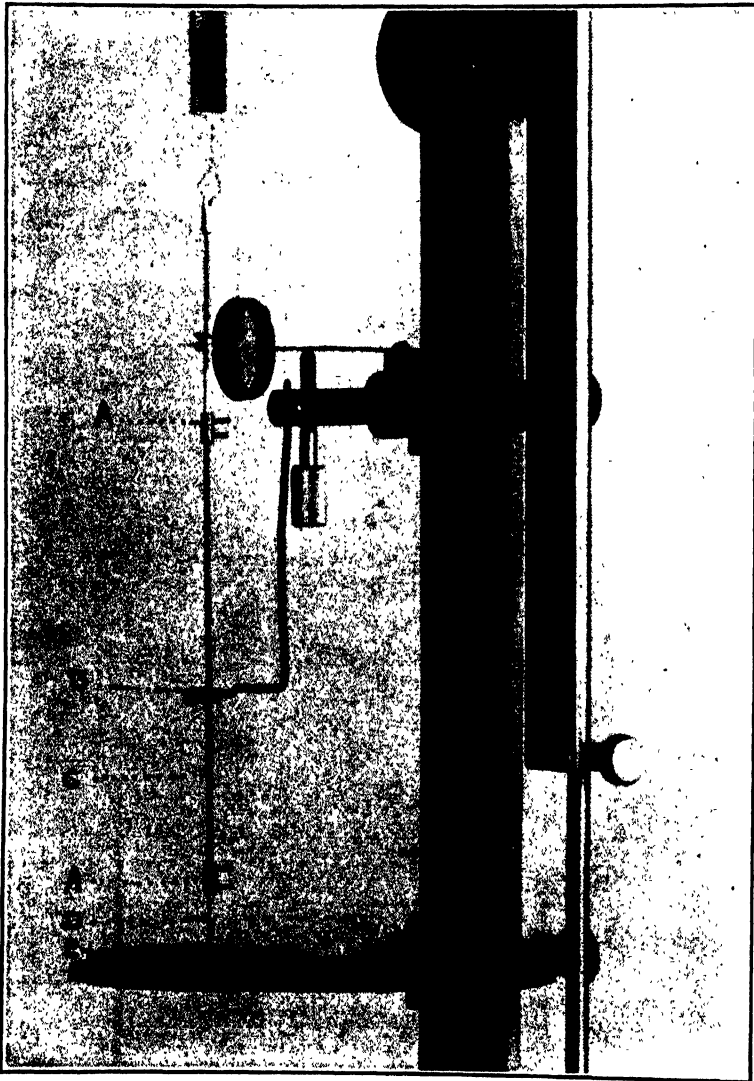


FIG. 4. Modified Jolly balance. A. Coupling. B. Guide. C. Brass rod. D. Phonograph needle with specially ground point. E. Paraffin block.

opment of the organism, however, depends upon the variety concerned. Certainly the germ tubes are able to enter some varieties without being able to develop beyond that point.

(Species of *Berberis* very resistant to puncture usually also are resistant to rust. This is well shown by *B. thunbergii* (immune), *B. chinensis* (resistant), and *B. lycium* (resistant). The reason for the inability of the germ tubes to penetrate seems to be threefold. In some varieties the outer walls of the epidermal cells are very thick and resistant to puncture. In other varieties there is a thick cuticle; and in still others the perpendicular walls of the epidermal cells are thick and the epidermal cells themselves are small, an arrangement which probably increases the resistance to puncture.)

(It has been shown quite clearly that the reason young leaves of barberry often are susceptible but become resistant with age is that the epidermal walls become increasingly resistant to puncture as the leaves grow older. This is shown clearly by *Odostemon repens* and *O. aquifolium*. The outer walls are thick, but easy to puncture. Later, however, they become very resistant to puncture, apparently owing to the production of a waxy exudate which becomes a thick, tough cuticle within about three days)

SUMMARY

1. The resistance of barberries to stem rust may be due either to morphological or to physiological causes.

2. No indication was found that the sporidial germ tube of *Puccinia graminis* entered an immune host like *Berberis thunbergii*.

3. The leaves of *B. thunbergii* (immune) have much thicker outer walls on the epidermal cells and are more resistant to puncture than those of susceptible species.

4. The difference between the resistance to puncture of one-day-old leaves of *B. thunbergii* (immune) and *B. vulgaris* (susceptible) is more than 24 times the probable error of the difference. One-day-old leaves of *B. chinensis* (resistant) have a puncture index intermediate between those of the susceptible and immune varieties.

5. The resistance of neither *B. brachypoda* nor *B. pruinosa* can be explained on the basis of resistance to puncture.

6. Immunity of *Odostemon repens* probably is partly physiological. However, the cuticle of both this species and *O. aquifolium* (resistant) becomes thick and resistant to puncture within a few days.

7. Those varieties whose leaves are characterized by thin and easily punctured outer walls of the epidermal cells were most susceptible.

8. That leaves of susceptible and resistant varieties become immune from puncture with increasing age can be explained by the thickening of the outer epidermal wall and the accompanying rapid increase in resistance to puncture.

9. The results obtained seem to indicate rather clearly that those species of *Berberis* which are very resistant to puncture usually are resistant to rust also. The converse, however, is not necessarily true. In addition to this morphological resistance, it appears that there also is a physiological resistance. While resistance to puncture, therefore, probably indicates real resistance to the rust organism and possibly may be used as a criterion for resistance, ease of puncture does not necessarily indicate susceptibility.

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A NEW ALTERNARIA DISEASE OF ONIONS (*ALLIUM CEPA* L.)

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This paper deals with an *Alternaria* disease which appears to be distinct from similar maladies of onions heretofore described. A very severe outbreak of the disease occurred during December, 1924, and January, 1925, in three different places in Porto Rico: in the central part near Barros, in the north near Vega Baja, and in the Experiment Station grounds at Rio Piedras. A study of the pathogene has been made, including its morphology, cultural characters and causal relation to the disease.

SUSCEPTS

The market onion (*Allium cepa* L.) and the false shallot (*Allium cepa* L., unnamed variety)² are the only species known to be affected by this disease.

It appears from field observations that all varieties of onions grown in Porto Rico are equally susceptible. These include the red, yellow, and white varieties of the so-called Bermuda onion. Plants of all ages appear to be susceptible but the disease is apparently most destructive just before bulb formation has started. Of course much depends on the season, and it may be that the injury will be equally great on plants at any age under favorable seasonal conditions.

THE DISEASE

Name

Since the purple color of the lesions is characteristic of the disease and distinguishes it from other known leaf diseases of the onion, the name "purple leaf spot" is here proposed for it.

¹ Presented to Cornell University in partial fulfillment of the requirements for the degree of Master of Science.

The writer gratefully acknowledges the assistance and suggestions of Dr. Mel. T. Cook, Plant Pathologist, Insular Experiment Station, Porto Rico, under whose direction this work was begun. He is especially indebted to Professor H. H. Whetzel, of the Department of Plant Pathology, Cornell University, under whose direction the work has been completed, for his suggestions and criticisms in the final preparation of the paper.

² In Porto Rico and other countries a race of *Allium cepa* with small multiplier bulbs has been called shallot. It does not bear any resemblance to the true shallot. It is here referred to as false shallot as suggested by L. H. Bailey.

HISTORY AND RANGE

Three similar diseases of the onion have been reported. The so-called leaf blight, caused by *Macrosporium parasiticum* Thüm., generally has been regarded as a secondary malady. There is moreover no experimental evidence of an independent causal relation of the fungus involved. The symptoms produced on the suscepr following the entrance of the fungus through wounds are distinct from those of the disease in hand. It is distinguished by large dark to brown, sometimes light, spots (14, p. 235), while the disease here studied is characterized by purple lesions.

Another related disease is that produced by *Macrosporium porri* Ellis. The following quotation from Thaxter (15, p. 161) gives a good symptom picture of this malady: The disease "occurs commonly upon seed though less frequently on market onions. . . . The spots are paler than those which characterize *M. parasiticum*; usually more circumscribed and less inclined to inflict injury by the rotting and breaking of the seed stalk at the diseased point." The purple leaf spot causes a distinct rotting of the tissues, as will be pointed out later. Again, the characteristic coloration of the lesions and the fact that it occurs on the foliage of market onions are points which further emphasize its distinctness from the leaf spot caused by *M. porri*.

Ajrekar (1) reported a disease which was threatening the cultivation of onions in Bombay and which he thought probably due to a species of *Macrosporium*. The following year he reported the same disease as a leaf spot and blight, attributing it to *Alternaria* sp. (2). He holds it to be favored by the presence of thrips and states that the fungus is a wound parasite. He finds red varieties less susceptible than the white. There is no description given of the disease. It is doubtful that the purple leaf spot is the same as Ajrekar's blight. Evidence will be presented later to show that the pathogene with which we are concerned in the present case is not necessarily a wound parasite. It has already been stated that red varieties are as susceptible as the white.

The purple leaf spot was first reported simultaneously by extension agents from Barros and Vega Baja, Porto Rico, during the month of December, 1924. Material for study from those places was received at the Experiment Station at Rio Piedras during the same month. Early in January, 1925, the disease appeared in an experimental plot at the Experiment Station and furnished excellent material for further study.

Economic Importance

The disease caused considerable damage to the 1924-25 crop in Barros and Vega Baja and greatly reduced the yield. In one instance the writer observed the almost complete destruction, during rainy weather, of a large planting where the disease appeared before the bulbs had begun to form.

The disease may be expected to be one of increasing importance in the island since the onion promises to become a more important crop in Porto Rico in the near future than it has been up to this time.

Symptomatology

The disease attacks both the leaves and flower stalks. The first symptoms are numerous tiny, white, circular or irregular spots, less than one millimeter in diameter. These gradually increase in size until in advanced stages the diseased areas cover several square centimeters of surface. The daily progress of the lesion into the healthy tissues is marked by a new zone of freshly discolored tissues. As the spots increase in size, they become oval-shaped or irregular and the incipient white color eventually changes to violet.³ Later stages of development show the central portion of the spots changing to a Pompeian red, dark Indian or Perila purple, immediately surrounded by a pale yellow orange to salmon band beyond which is a pale green zone. Dull violet-black zones within the lesions are also to be observed. In many instances these colors blend into different shades, spreading over the whole surface of the lesion. The dark purple color is the most distinctive symptom of the disease. A distinct yellowing usually extends from both ends of the spots, often reaching the tips and bases of the leaves. In very old spots the purple or violet color gradually fades out, and a pale ochraceous-buff develops, the center retaining some shades of purple. Similar lesions are produced on the flower stalks. The lesions on the latter cause girdling in most cases, and as a rule the stalks are destroyed before the seeds are mature.

Brown conidia of the pathogene soon appear in abundance, borne at the end of dark colored conidiophores on the surface of the lesions, giving a brown tinge to the spots.

The spots on the false shallot are similar to those on the onion but they are smaller and the color is somewhat paler. The purple color is not so pronounced as on onion leaves.

Etiology

Classification and identity of the causal organism

Taxonomic relations. The original basis for distinguishing between the genera *Alternaria* and *Macrosporium* is the manner of spore production, *i.e.*, whether they occur singly or in chains. Those forms producing spores in chains have been generally placed under the genus *Alternaria*, while those occurring singly, irrespective of morphology, have been considered to

³ Throughout this paper references to colors are based on Ridgway's "Color Standards and Nomenclature."

belong to *Macrosporium*. That there is on this basis no sharp line of demarkation is now a well-established fact. There are forms which agree in every other respect with the morphologic characters of *Alternaria* but do not ordinarily produce spores in chains. The question then arises as to the genus under which this particular species is to be included.

Elliott (4) published an account of his investigations on these two genera. He states that "all spores of the obclavate, cuneate, or ovate form produce chains of spores under favorable conditions." He also states that "there is no doubt that all of the species with these types of spores belong to the genus *Alternaria*, that most of the species named under *Macrosporium* belong to this genus, and can be recognized as such by the descriptions given of their spores." He adds further that "where species of *Alternaria* have been described the spores have been more or less elongated and pointed at one end." This fungus appears to exhibit all the morphologic features of an *Alternaria* as that genus is defined by Elliott. In referring the species to this genus the writer has, therefore, accepted Elliott's opinion that "all obclavate, cuneate, ovate, pointed or beaked spores belong to *Alternaria*."

As to the specific identity of this pathogene, it can only be pointed out that it apparently differs morphologically from other so-called *Macrosporium* and *Alternaria* species heretofore described as occurring on onions. A comparison of the description of the fungus, given below, with that of *Macrosporium parasiticum* Thüm. (13) shows them to be quite distinct. The latter has oblong-ovate to depressed, rotund spores always rounded at both ends as contrasted with the oblong-clavate and beaked spores of this species. Moreover, the spores of *M. parasiticum* are minutely warted (8, p. 3, pl. III) while those of this species are smooth. It is clear that they are not even generically identical, *M. parasiticum* being a true *Macrosporium* in the sense of Elliott.

While *M. porri* Ellis is undoubtedly an *Alternaria*, it has much smaller spores than those of this species. Moreover, its spores have simple beaks, judging from the description by Saccardo (13) and the illustrations by Thaxter (15, figs. 40-43). A large number of the spores of the species under consideration possess compound beaks. It may be identical with the *Alternaria* which Ajrekar (1, 2) believes to be the cause of an onion leaf blight in India but, as he gives no description of his fungus or the symptoms caused by it, one cannot assume them to be identical. On the basis of such evidence as is available it seems wisest to call the purple leaf spot pathogene a new species which is here designated *Alternaria allii*.

***Alternaria allii* sp. nov.** Mycelium in the lesions hyaline or brown; in culture white to smoky, reddish or fuliginous, blackish-green or olive; 4-10 μ

thick in the lesions, sometimes attaining a thickness of $18\ \mu$ in culture; composed of smooth, septate, short, simple or branching, subfasciculate hyphae; in culture media, cells often much constricted at the septa, frequently separating smoothly at these constrictions, containing distinct refractive droplets; chlamydospore-like bodies and gemmae present in cultures. Conidiophores arising through the stomata or breaks in the epidermis as terminal branches of hyphae; solitary or fasciculate; erect or decumbent; septate; fuliginous to dark brown or even brown olivaceous; blunt at the tips; $20-180 \times 4-18\ \mu$, basal cells or segments usually wider than the terminal portion of the sporophore. Conidia borne singly at the apex of the conidiophore; elongate-clavate, apically attenuate to form a long beak which is often branched; multiseptate, muriform; $105-320 \times 12-24\ \mu$; at first hyaline, brown or dark olivaceous when mature; the broad base of the spore 6-12 septate with one or two longitudinal septa in the middle segments of some; slightly constricted at septa; beak continuous or several-septate; germinating very readily in water; spore production in culture media exceedingly scanty or none.

Hab. On leaves of *Allium cepa* L. in Porto Rico.

Pathogenicity of Alternaria allii. Microscopic examination of the lesions shows the constant association of a fungus of the *Alternaria* type. The affected tissues are invaded by a ramifying, septate, brown mycelium from which arises over the necrotic area of the spot an abundance of typical large muriform spores (Plate III, 11-22).

Cultural characters. Pure cultures were obtained from typical spots on the leaves of onions by plating out spores in agar or by tissue plantings.

All carbohydrate media were sterilized in the Arnold steam sterilizer for one hour on three consecutive days. All other media were autoclaved for 30 minutes at 20 lbs. pressure. The pathogene was grown six times in each of the culture media, using pedigree cultures, and detailed observations were made daily on changes which occurred. All cultures were grown at room temperature, which ranged around 78° F. during the period when this work was carried out.

On oatmeal agar the fungus makes a very profuse growth. The mycelium is dark with a reddish to violaceous tinge. The substratum is cadmium-orange. Masses of aerial mycelium later become amethyst-violet to hyacinth-violet. A dusky violet blue tint is often observed in the mycelial growth.

On Cook's II agar⁴ the mycelium is profuse, smoky white to dark dull gray. The substratum is black except in the region lying just below the

⁴ Preparation: 20 gms. agar agar; 10 gms. peptone; 20 gms. glucose; 0.5 gm. dipotassium phosphate and 0.5 gm. magnesium sulphate; water 1,000 cc.

outer growing ring of the mycelium which is light-brown, changing later to hazel brown.

On Czapeck's agar⁵ the organism makes excellent and extremely rapid growth. The aerial mycelium is profuse, mostly mineral red with a smoky white outer growing zone. The substratum is of a more or less Mars violet color.

On nutrient agar⁶ growth is moderately fair; aerial mycelium brown or gray. The substratum does not change in color.

On non-nutrient agar growth is very poor, scanty and loose; aerial mycelium white.

On saccharose (3 per cent) agar growth is fairly good; aerial mycelium loose, whitish, and the substratum sulphine yellow.

On dextrose (3 per cent) agar the substratum is olive green; aerial mycelium greenish; growth fair.

On lactose (3 per cent) agar growth is fair; substratum olive green; aerial mycelium loose, dark.

On dextrine (3 per cent) agar the substratum is aniline yellow; aerial mycelium whitish; growth fairly good.

On maltose (3 per cent) agar the substratum is yellowish-oil; aerial mycelium greenish, loose.

On cornmeal agar the growth is profuse; aerial mycelium ochraceous-buff to yellow ochre with violaceous spots scattered throughout the surface; substratum Mars violet. A few spores appeared at the end of 4 days.

On Thaxter's hard potato-dextrose (2 per cent) agar aerial mycelium is profuse, varying in color from ochraceous buff to olivaceous black and an intermediate violet color. A few spores were found at the end of 3 days.

On hard potato saccharose (2 per cent) agar the outer growth of mycelium is yellow ochre; older inner mycelium aconite violet; substratum Morrocco red to blackish. A few spores were found at the end of 3 days.

On hard potato-lactose (2 per cent) agar the mycelium produces abundant whitish aerial tufts, although the rest of the aerial mycelium is dark gray; substratum vinaceous; tawny under young growth and black under the old growth.

On hard potato-maltose (2 per cent) agar the growth is similar to that on potato-saccharose agar but spores are not produced; aerial mycelium profuse, violaceous to purple.

A comparative study of the rate of growth on sugar and sugar-starch media in petri-dish cultures showed at the end of 7 days for lactose and dextrine (3 per cent) agars, a thallus diameter of 6.5 cm.; for saccharose,

⁵ Prepared by adding 10 gms. agar agar to 500 cc. Czapeck's solution.

⁶ Prepared by adding 10 gms. agar agar to 500 cc. bouillon consisting of 2.5 gms. Liebig's beef extract, 2.5 gms. sodium chloride and 500 cc. distilled water.

dextrose, maltose (3 per cent) and cornmeal agars a diameter of 7.5 cm.; for potato-saccharose, potato-dextrine and potato-maltose (3 per cent) agars a diameter of around 8.5 cm. while on potato lactose (3 per cent) agar the thallus reached a diameter of 9 cm.

On scales of yellow onion bulbs cut in pieces and steamed in petri dishes in the autoclave, growth is excellent, producing a dense white mass of mycelium at the end of two days. This later changes to a rather olivaceous or reddish color. The color of the scales changes to a reddish or olive color. This color reaction approaches that observed on the tissues of the susceptible under natural conditions in the field and confirms the opinion that such color effects are entirely due to this fungus.

On pieces of onion leaves steamed in test tubes in the autoclave, the fungus produces abundant and dense masses of white mycelium.

In bouillon (-15 Fuller's scale) growth is fairly good and abundant smoky gray mycelium is produced.

Czapeck's solution gives the best growth of all liquid media tried. A dense mycelial felt covers the surface of the liquid. The superficial mycelium is violaceous, the submerged is dark brown to black.

On Cohn's solution growth is fair, mycelium whitish.

On onion decoction the mycelium is dense and abundant as in Czapeck's solution. A purplish tinge is noticeable in the superficial mycelium while the liquid becomes filled with dark brown mycelium.

Summary of cultural characteristics on these media:

1. The organism makes profuse growth in all carbohydrate media.
2. Growth is best on oatmeal agar, cornmeal agar, Czapeck's agar, Cook's II agar, and potato-sugar media.
3. Growth is relatively poor on sugar media not containing starch or proteid.
4. Growth on onion tissues and decoctions is fairly good.
5. The fungus causes a change in color of the sterilized onion scales, which is similar to that produced on the susceptible under natural conditions.
6. Best growth in liquid media was observed in Czapeck's solution.

In cultures, especially in carbohydrate media, the pathogene itself exhibits marked color variations. Ravn (12, pp. 101-327), in studies of the genus *Helminthosporium*, shows that the plasma and cell-walls may be blackish-green, grayish-brown, or entirely black as in the genus *Alternaria*. Teodoro (14, p. 242) working with *M. parasiticum* Thüm., states that the mycelium is at first hyaline but later assumes a smoky white to light, yellowish-brown color and later becomes deep brown and greenish. Similar colorations have been observed in the mycelium of *A. allii* in culture.

The smooth mycelium branches easily and profusely in water and culture media, anastomosing into a compact, intricate, heavy white mass. Neighboring hyphae sometimes anastomose.

Drops of slightly-colored liquid separate from the body of the fungus and collect on the surface. The nature of these drops was not investigated. Chlamydospore-like bodies and gemmae have been found in old cultures, a character observed in *Helminthosporium* by Mitra (7).

Abnormalities have been observed in the mycelium under various treatments. Ten-day old cultures on potato-dextrose agar were flamed to produce a slight injury to the superficial mycelium. The plates were then set aside and aerial mycelium was allowed to grow. Abnormal swellings in the injured hyphae were induced, this occurring very frequently in the end cells, in some cases suggesting the early stages of spore formation. In other cases small, more or less irregular or elongated structures were seen to arise on the sides of cells.

Effect of environmental factors on growth in culture. Experiments with different concentrations of acid and alkali demonstrate that *Alternaria alli* makes its maximum growth in media of slightly acid reaction. It was also found that the fungus will tolerate larger concentrations of alkali than of acid.

To ascertain whether light has any effect on growth, two sets of plate cultures on Czapeck's agar were arranged as follows: 10 plates were wrapped in heavy black paper of the kind used for photographic purposes (each plate separately) and were kept in the dark at room temperature. Another set of the same number was kept in the light. In the plates kept in the dark there was slightly less aerial mycelial growth but color relations were much the same as in case of the plates kept in the light. The difference is insignificant or almost negligible.

To determine the effect of quantity of medium on growth, two sets of six plates each were poured with Czapeck's, Cooks' II, and oatmeal agars, one set containing 12 cc. and the other 24 cc. of medium per plate respectively. Transfers were made from pure cultures to the center of each plate. The plates were kept in the light at room temperature. The results were recorded daily for a period of seven days.

It was found that growth is uniformly greater in the larger quantity of medium. Aerial mycelium was much denser, producing a heavy hyphal mat over the surface of the plates containing the double quantity of medium. No differences in color of mycelium or substratum were observed.

To determine the effect of inhibitory influences on production of spores and character of thallus growth, transfers of the fungus from pure culture were made to Cook's II agar plates making the transplant at the center of the plate. Three transplants of bacteria (species not determined) and one

of *Aspergillus* sp. were planted on the border of the agar close to the edge of the plate. It was observed that the thallus growth of the *Alternaria* was inhibited and checked in the direction of the other plantings. The medium under the regions where the fungus was approaching these plantings became cadmium to lemon or chrome in color, contrasting with the dark gull gray of that under the rest of the thallus.

• To determine the effect of moisture on growth, rice tubes were prepared using a small quantity of water in one case, in another using double the quantity. In the same way onion leaves were steamed in test tubes. The fungus was grown for six days in these tubes and the results were observed. In tubes with the least water the growth was rapid but limited. In tubes with the larger quantity of moisture growth was dense, profuse, heavy with abundance of white aerial mycelium.

A great variety of culture media has been used in an effort to obtain spores of the fungus under purely artificial conditions. The methods employed by Rands (10) and Kunkel (5) have been tested. Teodoro (14, pp. 242, 267-268) was successful in obtaining conidia in potato-dextrose agar with *Macrosporium parasiticum* Thüm. All attempts to induce conidial production have been unsuccessful. In these studies a few spores of *A. allii* have been obtained in 3- or 4-days old cultures in oatmeal, potato-dextrose, and hard potato-saccharose agars but spore production soon ceased and was never abundant. The only explanation which suggests itself for this phenomenon is that the mycelium of this species exhibits an excessive tendency toward hyphal growth at the expense of spore production. This is especially true of media with high moisture content. There is undoubtedly a delicately balanced relation between spore production and air humidity over the growing colony.

The spores produced in cultures are considerably smaller than those which occur under natural conditions. They very rarely exhibit the characteristic long beak or isthmus, this remaining more or less rudimentary. The conidia germinate in cultures as soon as they are produced.

Summary of effect of environmental factors:

1. The fungus tolerates larger concentrations of alkali than of acid. Best growth is obtained in slightly acid media.
2. The amount of light does not have much influence on thallus growth.
3. There is much more growth with the greater amount of medium.
4. Colonies of other fungi and bacteria show inhibitory influences on the growth of *Alternaria allii*.
5. Large quantities of moisture in the medium increase the abundance of white aerial mycelium.

Infection experiments. The inoculum used throughout the infection experiments was mycelium from single spore cultures used in the preceding studies and spores taken from natural lesions.

Six healthy plants growing in clay pots under field conditions were sprayed with a spore-suspension of the fungus. The pots were then covered with bell jars lined with moist filter paper. Two days later the first symptoms of the disease appeared as small, whitish necrotic areas. A second set of six plants was sprayed with a suspension of bits of mycelium from 6-day old cultures and covered with bell jars. The symptoms of the disease appeared on the second and third days. A third set of plants was inoculated by transferring spores of the fungus to needle-pricks made on the surface of the leaves. A rapid dying of the adjacent tissues was observed on the second day. A fourth set of plants was inoculated through needle-pricks on the leaves with bits of mycelium from 6-day old cultures of the fungus. Dying of the surrounding tissues was observed on the second day. Check plants wounded but not inoculated showed no further evidences of injury.

Similar inoculation experiments were conducted in the greenhouse. The fungus was repeatedly reisolated from lesions produced by artificial inoculation.

These experiments together with the constant association of the fungus with the lesions establish beyond doubt the ability of *Alternaria allii* to cause the disease of onions under consideration.

Inoculation with spore suspensions gave slightly less prompt infection than that with growing mycelium from cultures. Infection was found to be most prompt when the inoculum was applied to wounds in the leaves.

Life History

In Porto Rico, onion seed is sown in seed beds and the seedlings transplanted into the field. Seed beds are generally started during the latter part of August, through September, and even during early October. Transplanting is done during October and early in November. At this time there is heavy rainfall in some of the onion districts and moderate showers in others. The average period of growth after setting of transplants in the field is three and one-half months under normal conditions (9). The harvesting season begins in December and lasts through January.

This pathogene has a very simple life history, living for the relatively short period of pathogenesis on the green leaves and seed stalks of the onion, and continuing its existence as a saprogene on the debris of its suspect after the death of these organs.

The primary infections usually occur just prior to bulb formation, about the time the plants show the greatest vigor. The source of inoculum is most probably the debris of diseased onions left on the field from the previous crop, as onions are commonly grown year after year in the same field.

Conidia produced during the previous season do not appear to serve as inoculum for the primary cycles. This is indicated by germination studies in which it was found that spores germinate promptly after they are produced, whenever moisture is present. This is further confirmed by the desiccation experiments from which it appears that the conidia do not withstand much drying and so soon lose their viability.

In the experiments to determine the effect of desiccation on the vitality of the spores, four drops of a spore suspension in distilled water were transferred to each of 60 glass slides. The water was then evaporated by means of an electric fan. The slides were divided into two lots, A and B, of 30 slides each. Lot A was placed in a moist chamber with the humidity maintained as near saturation as possible. Lot B was placed in a dry chamber, *i.e.*, at the prevailing humidity of the laboratory air. The room temperature was around 78° F. during the period of this experiment. Each day after these experiments were initiated, one slide from each lot was removed and a drop of sterile distilled water placed over each of the four spore smears on the slide. These were then placed in moist chambers to germinate. One hundred per cent germination occurred in slides from lot A during the first 7 days and in lot B during the first 5 days. Germination rapidly dropped to 50 per cent in both lots by the 12th day. On the 15th day lot A gave a germination of 33 per cent while lot B gave but 10 per cent germination. No germination was obtained from lot B after the 18th day, while lot A continued to give a rapidly declining germination until the 27th day, after which the spores would no longer grow. It is thus evident that if under the ordinary dry conditions of the laboratory spores are killed within 18 days, in the hot sun of the open fields they probably succumb in a very short time. Even a saturated atmosphere will not keep them alive for a month. Periods of high humidity under field conditions, while frequent, are usually of relatively short duration due to the trade winds which blow daily.

Since but one crop of onions is grown during the year, spores produced on the lesions on living plants will all have perished in one way or another before the next crop is growing. So only those conidia which are produced from mycelium in the old debris can serve as primary inoculum.

These conidia are transferred to the infection courts by spattering rains and possibly by thrips. Conidia may also be blown by the wind but this is probably of rare occurrence in the case of primary inoculations. The leaves are the only infection courts.

Conidia deposited on the leaves soon germinate if a film of water is present. The germ tubes enter through the stomata, and through wounds made by thrips (*Thrips tabaci* Lind.) or by other agents. A stomatal vesicle has not been observed to be produced by the germ tubes. Soon after entrance

the germ tubes branch in all directions and grow intercellularly. Incubation is completed and invasion accomplished in a few hours.

The first evidences of infection appear in from 1 to 4 days as small bleached or whitish spots. The mycelium branches and spreads in all directions through the tissues, killing all the adjacent cells. Growth and development of the pathogene is very rapid during the early stages of infection, especially when the plant is in an actively growing condition. The progress of the pathogene is accompanied by yellowing and bleaching of the invaded tissues, which is brought about by the destruction of the chloroplastids. The necrosis of the chloroplastids is followed by plasmolysis of the cytoplasm and by oxidation discoloration of the cell walls and cell contents.

Conidiophores bearing conidia begin to develop over the necrotic area of the lesion about the fifth day after the first symptoms appear. The leaves bearing these primary lesions soon shrivel and dry. They are shortly broken away by the wind or in cultivation and fall to the ground, where the fungus continues a saprophytic existence for a time, producing conidia for further infections.

Conidia produced on the primary lesions serve as the inoculum for secondary infections. They are produced in great numbers and are readily detached from the conidiophores. They are carried to the infection courts by the wind, spattering rain, and probably by thrips. Since the distance between plants in the field is never greater than 10 by 10 inches, spattering rain is probably the chief agent in conveying the inoculum. However, strong winds bring healthy leaves in contact with diseased ones and thus they become inoculated, especially when wet with rain or dew. In these ways the fungus is rapidly disseminated throughout the field.

Spore germination has been carefully studied. The conidia for these studies were taken from natural lesions in the field, since, as already pointed out, conidia have not been readily obtained in culture. Spore suspensions were made by scraping conidia from the lesions and transferring them to 5 cc. of the liquid medium in which they were to be germinated. Germination was studied in tap water, rain water, distilled water, beef bouillon, Czapek's solution, Cohn's solution, onion leaf decoction and onion scale decoction. All studies were made in inverted drops of these media in Van Tieghem cells held at room temperature. The spore suspensions were started about 9 A. M. and were examined first at 5 minute intervals, later every 2 minutes.

All spores began to germinate at the end of 21-45 minutes. Just before germ tubes begin to appear the contents of the cells in some cases contract. The constriction between the cells of the conidium becomes more pronounced due to increased turgor of the cells. This is especially true in the case of

the cells of the beak (Plate IV, 26 and 28). The first definite indications of germination are slight protrusions in the cell walls which rapidly develop into definite germ tubes. A septum is soon formed in each tube a short distance from the germinating cell. At the end of three hours practically every cell of the basal part of the spore has usually germinated, as well as some of those of the beak (Plate IV, 26 and 28). The germ tubes by this time are all abundantly septate (Plate IV, 28). The width of the tubes at this time is from $3.4\ \mu$ to $4.3\ \mu$, and branching has already begun.

A comparative study of spore germination as it progressed in the different media above listed gave some results probably worthy of record. The length of the germ tubes at the end of 2 hours was approximately the same in all media except distilled water, in which they were appreciably longer. At the end of $4\frac{1}{2}$ hours they were all about the same length except in beef bouillon, where they were distinctly shorter. In tap water, rain water, distilled water and Cohn's solution there was little tendency to branching of the germ tubes even at the end of 6 hours, although their length was as great as in the other media. This probably was due to a lack of available nutrients in the media, since in those cases growth is largely dependent on the food stored in the spore.

In bouillon, Czapeck's solution, and in decoctions of onion leaves and scales, the germ tubes were profusely branched and abundantly septate at the end of 6 hours, indicating the absorption and use of the nutrients in these media.

In onion scale decoction and onion leaf decoction, germination was slightly slower than in non-nutrient solutions and showed a tendency to marked septation. Trials to determine the effect of different strengths of the onion leaf or scale decoctions on germination were conducted, using varying amounts of tissue in a given amount of water. The results show a slight inhibitory or deleterious influence of the higher concentrations.

On solid media it was observed that in general the germ tubes are wider, shorter, and more constricted at the septa, exhibiting profuse branching.

Invasion of the uninjured leaf by the germ tubes appears always to be by way of the stomata. Repeated observations fail to show any case of direct penetration through the epidermal cells. The invading hyphae spread through the intercellular spaces of the leaf. Actual penetration of the cells themselves has never been observed. Hyphal tips emerge through stomata or in some cases rupture the epidermis to give rise to the conidiophores.

Various types of conidiophores have been observed in this species. Among the most common is a short septate, finger-like conidiophore borne at the end of a fertile hypha singly or in groups (Plate III, 3, 7 and 8 and Plate V, 33-39, 46-47). It is hyaline to light or dark brown and blunt at the tip. The end cells of the vegetative hypha become enlarged or swollen

and constitute the basal cells from which the conidiophore arises. The number of these swollen tips varies from one to four or five at each point of emergence. This type of conidiophore has extremely heavy walls, and like the mycelium it becomes filled with refractive drops or is abundantly vacuolate.

The second type of conidiophore is characterized by thinner walls, is longer and slightly narrower (Plate III, 1-2, 4-6). It occurs in abundance in very old lesions, and the basal cell is not so swollen as in the preceding type. It is closely septate and dark brown in color. Old conidiophores of this type show ovoid to globular or irregular small cells at their ends. This probably indicates the formation of conidia successively on a conidiophore, each corresponding to a detached conidium (Plate III, 9). Clusters of conidiophores usually arise from one, two or three swollen cells in the fertile hypha (Plate V, 60).

Conidia develop from the uppermost cell of the sporophore. At first they are small, hyaline, one-celled and long-ovate (Plate V, 50). The next step is marked by a slight growth in width and pronounced growth in length; septation begins. The walls now become slightly colored. At this stage conidia are two- to three-celled, and the apex becomes slightly attenuated (Plate V, 51-53). The base becomes enlarged and the apex greatly elongated. The base now becomes several-celled and light brown, the protoplasm taking on a more granular appearance while the beak remains hyaline. The spores vary in form, some being club-shaped while others are flask-shaped (Plate V, 54-58). The number of cells in the base increases rapidly, the spore takes on a decided brown coloration throughout the large basal portion and the apical cell becomes fully elongated into a hyaline beak or isthmus which is often branched (Plate V, 59), though there are always some spores with simple beaks only (Plate III, 14, 16-21).

A large majority of the older conidia possess compound or branching beaks. This peculiar branching of the beaks, which has been observed in *Alternaria radicina* Meier, Drechsler and Eddy (6, p. 163, fig. 2) and in *A. solani* (E. and M.) J. and G. by Rands (11, fig. 4) seems to occur once or twice in individual spores, resulting in two or four branches, as the case may be, arising from a single central beak (Plate III, 13, 15, and 22). Even six branches may be encountered in some cases. The occurrence of branched or compound beaks in these conidia presents a sharp contrast between this species and others of the genus *Alternaria* where conidia are borne in chains. Catenulation of spores has been sought in vain, both in nature and in culture. It evidently does not normally occur, if at all, in this pathogene.

The middle portion of the spore is usually wider and the cells are more numerous in this region. The walls of the spores are thick, as are the walls of the conidiophores. Two types of conidia differing as to size were observed in some of the lesions, some decidedly smaller than the usual large

type (Plate III, 16-21). Isolations from these spores were made. Isolations were also made from another specimen showing conidia of the usual large size (Plate III, 11-15). Field inoculations were properly carried out using both strains. Both produced similar lesions. Later examinations revealed the fact that each strain was capable of producing spores of either size. It may therefore be concluded that they belong to the same species and are only variations. What may be the conditions bringing about these variations in spore size has not been investigated.

Successive secondary cycles continue the destruction of new foliage as fast as it develops, preventing the filling or development of the bulbs. The blighted leaves shrivel and fall to the ground, where the fungus continues its activities under favorable moisture conditions until the next crop of onions is planted and presents growing foliage for the initiation of another period of pathogenic activity.

Epiphytology

The ideal field conditions for epiphytotic development of the disease appear to be warm weather with occasional rains. Just such weather conditions prevailed during January, 1925, in one experimental plot at the Experiment Station, and the disease was most destructive. There was a very vigorous, luxuriant growth at the time the disease first appeared. In seven days the pathogene had infected all the plants. Light afternoon rains favored the development of the pathogene, which soon destroyed the greater part of the foliage. Heavy losses were reported from another section of the northern part of the island where conditions were similar to those prevailing in the Experiment Station grounds. The disease made a violent attack during December, 1924, in the central part of the island, where the humidity of the air was high. Continued rains occurred and there was little sunshine.

The relation of light to spore germination and its bearing on time of invasion of the leaves was studied. All tests were made with spores in hanging drops of distilled water in Van Tieghem cells. Germination at night occurred promptly in 27-40 minutes and was vigorous and normal. In tests made at 7 A. M. in the shade, the spores began to germinate in 21-35 minutes; septation and branching followed rapidly. In short, early morning germination paralleled in every respect that which took place at night. To determine the effect of direct sunlight on spore germination, parallel sets of spore suspensions were set up at 8 o'clock in the morning, one in the shade, the other exposed to the direct rays of the sun. Those in the shade began to germinate in 21-32 minutes; those in the sunlight in from 27 to 43 minutes. Three hours after the spores were placed in suspension, measurements of germ tubes were made in each set. Those in the shade averaged 166μ , while in the direct sunlight the maximum was 9μ . It is evident therefore that, while diffused light has little or no effect on promptness or

vigor of germination, the direct rays of the sun markedly retard growth of the germ tubes or even prevent their formation. It seems clear, then, that spore germination and invasion of the leaf, or in other words incubational activity, occurs only during the night and early hours of the morning or during cloudy weather. Showers during the day followed by bright sunny weather is evidently not favorable to infection.

CONTROL

No field experiments on the control of this disease have been undertaken. Preliminary studies on the toxic effects of copper sulfate were carried out. Spore suspensions were made in solutions of copper sulfate in concentrations of 1:100, 1:1,000, 1:10,000, 1:100,000 and 1:1,000,000 in Van Tieghem cells. Hourly observations were made during the first day and the final results recorded at the end of two days. The spores germinated normally in dilutions of 1:10,000 and higher, but germination was almost completely inhibited at 1:1,000.

It would appear from these tests that *Alternaria allii* is relatively resistant to copper, the results indicating a susceptibility to copper poisoning about equal to that of *Alternaria solani* as determined by Doran (3, p. 535). While no tests of the fungicidal efficiency of copper in Bordeaux mixture against *A. allii* have been made, it is doubtful that it will prove effective, especially in view of the difficulties of making it spread and adhere to the glaucous surface of onion leaves. There would seem to be some promise of success from the use of copper dusts of high copper content, especially if the copper be in colloidal form.

Sanitary practices involving the removal and destruction of diseased crop debris promise little of practical value because of the rapidity with which the diseased leaves break and crumble as soon as they shrivel and dry. Rotation of the crop where that is feasible should be of some value in holding the disease in check.

SUMMARY

1. A new *Alternaria* disease of onions occurring in Porto Rico is described.
2. The market onion (*Allium cepa* L.) and the false shallot (a viviparous variety of *Allium cepa* L.) are affected.
3. The disease is characterized on the onion leaf by dark purple, oval-shaped or irregular spots.
4. The symptoms on the false shallot are essentially like those on the onion except for a paler color of the spots.
5. The pathogene, a typical *Alternaria*, is described as a new species *Alternaria allii*. It is characterized by the branched beaks of its conidia.
6. The cultural characters of the causal fungus on a variety of media were studied. Growth on carbohydrate media is excellent in general, being

better in starch-containing media than in media containing sugars alone. Color changes are induced in all solid media including sterilized onion leaves and bulb scales; also in onion decoctions. An acid reaction of the medium is more favorable to growth than alkalinity. Light does not affect rate of mycelial growth in artificial media. Thalli of certain other fungi and colonies of certain bacteria inhibit growth of *A. allii*.

7. All attempts to induce abundant spore production in pure culture failed. A few spores were observed in some cultures.

8. Infection experiments fully establish the pathogenicity of *A. allii* on the leaves of growing onions. Wounds are not necessary for invasion. Entrance of the germ tubes is normally through the stomata.

9. The conidia germinate in water in 21-45 minutes at ordinary room temperature as soon as mature. They are short-lived, being very susceptible to drying and direct sunlight. Germination and invasion apparently occurs at night or in cloudy weather and only in the presence of meteoric water.

10. The pathogene apparently lives over from one onion crop to the next as mycelium in onion leaf debris on the soil, from which a crop of conidia for the primary infection is produced.

11. The conidia are relatively resistant to copper sulfate, being able to germinate in a 1:10,000 solution. A 1:1,000 solution prevents germination.

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EXPLANATION OF PLATES

PLATE III

Conidia and conidiophores of *Alternaria allii*

- 1-2, 4-6, 10. Typical large conidiophores.
3. Young conidiophores borne at branched apex of a fertile hypha.
7. Conidiophores arising from a single fertile hypha underneath the epidermis.
8. Fasciculate cluster of conidiophores.
9. Old conidiophore showing cells formed at apex as a result of successive spore formation.
- 11-12. Conidia attached to conidiophores.
- 13-15, 22. Typical large conidia with branched beaks.
- 16-21. Small conidia with simple beaks.

PLATE IV

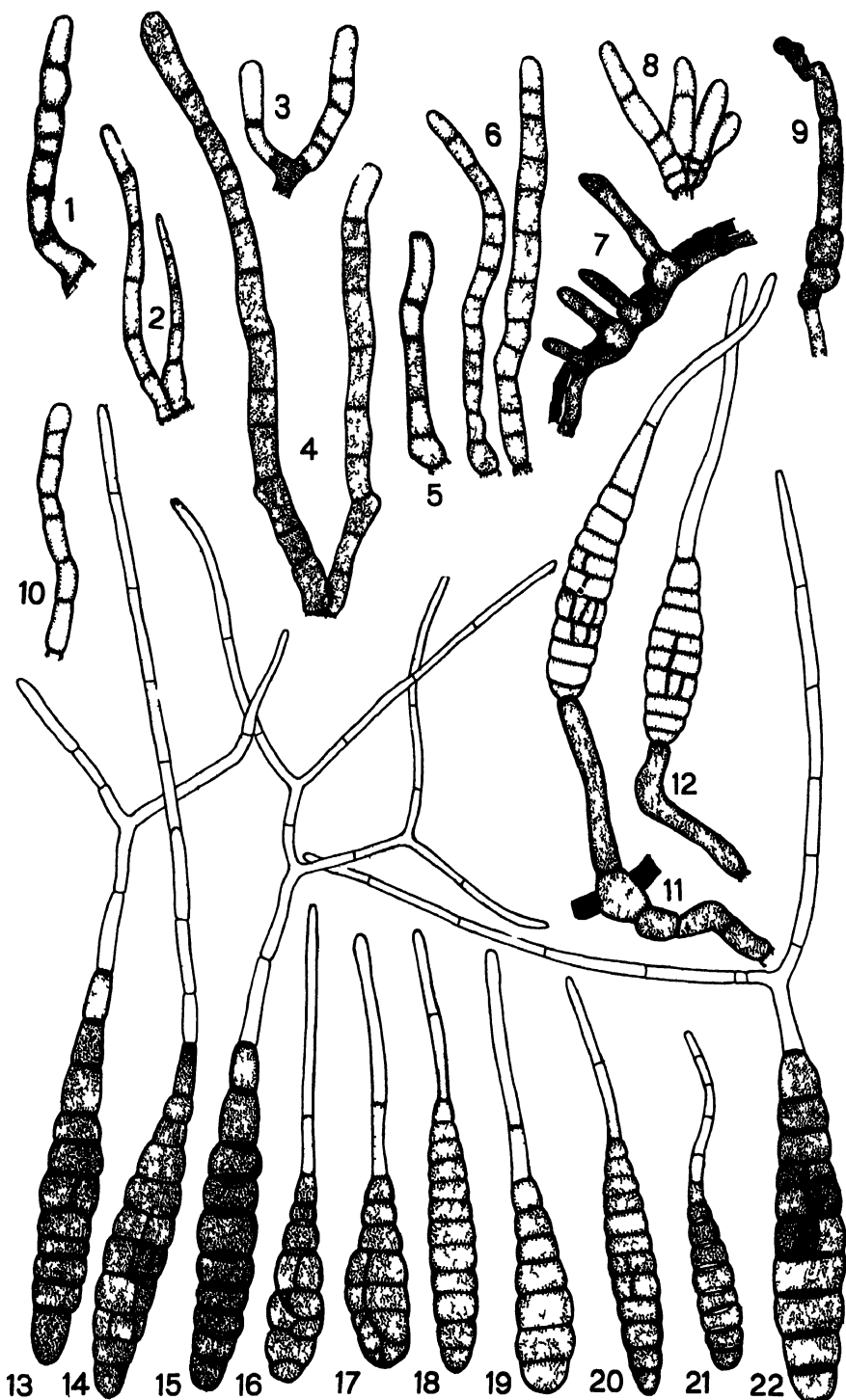
Germination of conidia of *Alternaria allii*

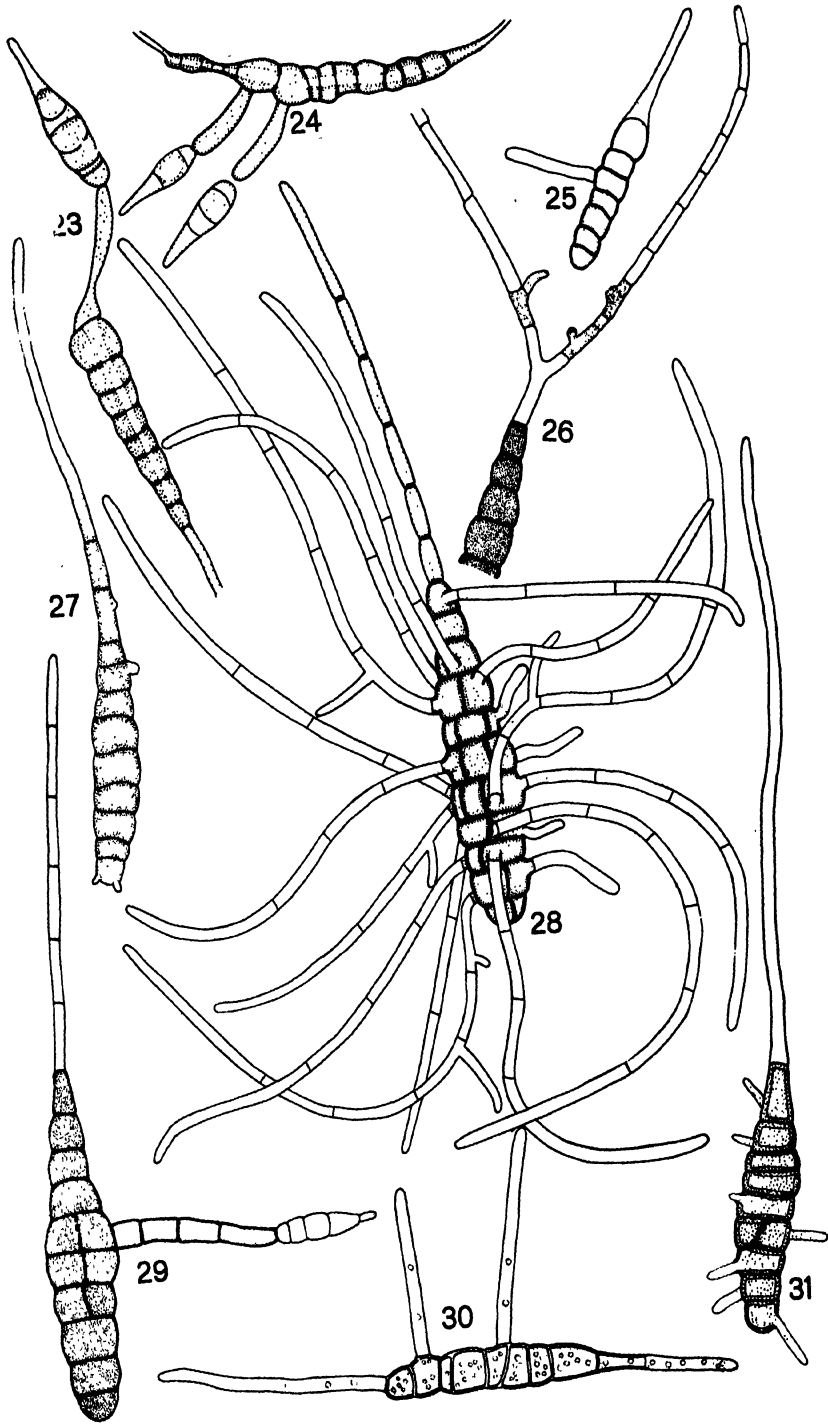
- 23, 24, and 29. Conidia germinating after a few days desiccation; showing production of secondary conidia at end of short, fertile germ tubes.
- 25 and 30. Young conidia germinating.
26. Cells of beak germinating. Note constriction at septa.
- 27 and 31. Extent of germination in distilled water after 21 minutes and 40 minutes, respectively.
28. Germinated conidium at the end of 3½ hours in distilled water. Note constriction at septa of beak.

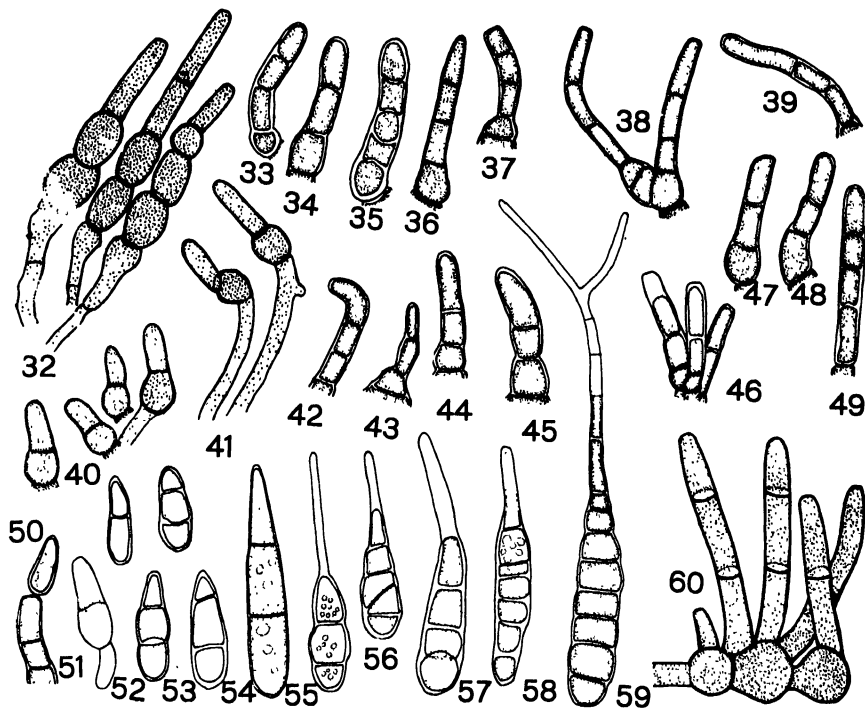
PLATE V

Development of conidiophores and conidia of *Alternaria allii*

- 32 and 60. Conidiophores arising from enlarged cells at the apices of hyphae.
- 33-49. Various stages in development of conidiophores.
- 50-54. Early stages in conidial development.
- 55-58. Primary septation of conidia and early stages in development of beak.
59. Final stage of conidial development, lower part of spore becoming septate and brown, beak becoming septate and branched.
61. Lesions of the purple leaf spot of onions.







ADDITIONAL HOSTS OF APHANOMYCES EUTEICHES, THE PEA ROOTROT FUNGUS

MAURICE B. LINFORD

Observations in Wisconsin pea fields and experimental plantings in the greenhouse have shown that the pea rootrot fungus, *Aphanomyces euteiches* Drechsler, has a wide range of potential host plants, and is not limited in its parasitism to *Pisum sativum*.

In the summer of 1925, narrow-leaved vetch was found with characteristically decayed roots containing oospores typical of this fungus in several fields of peas in two localities in Wisconsin. During 1926, seedlings of alfalfa, sweet clover, and an undetermined species of grass were found similarly diseased, and roots of barley and oats were found to contain oospores, apparently of this fungus. Several varieties of sweet peas were seen at McMillan, Michigan, much weakened by the attack of this pea rootrot fungus. Roots of red clover, alsike clover, white clover, and several weeds were examined in thoroughly infested fields but were not found to be affected.

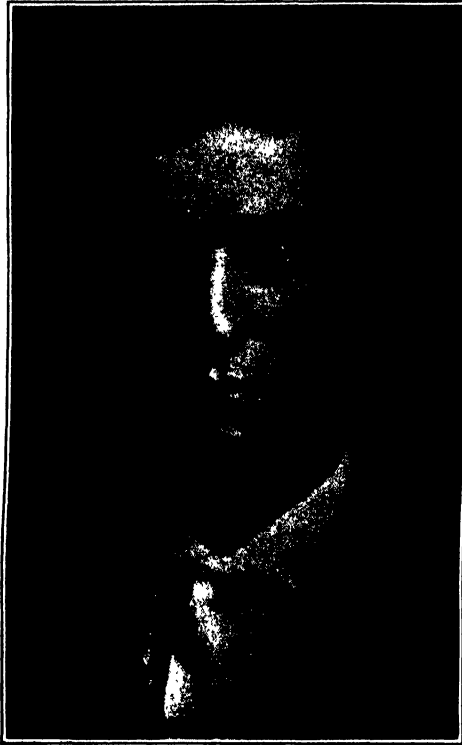
Of fifteen species of leguminous plants grown from seed in naturally infested soil (Colby silt loam) in the greenhouse in the winter of 1925-26, the following proved susceptible: *Medicago sativa*, *Melilotus alba*, *Lathyrus odoratus*, *L. latifolius*, *Lathyrus* sp. (native of northern Utah), *Vicia sativa*, *V. pannonica*, *V. monantha*, *V. gigantea*, *V. fulgens*, *V. ervilia*, *V. dasycarpa*, and *V. angustifolia*. Two species, *V. villosa* and *V. colcarata*, were not infected. An undetermined species of monocotyledonous bulb plant found by chance in the soil used in this trial also developed this rootrot disease. No pure culture inoculations have been made, but the morphology of the oospores of *A. euteiches* is sufficiently characteristic to make possible the rather positive identification of the parasite.

All of these hosts are relatively resistant in comparison with peas, as shown by lower percentages of plants infected, by less extensive invasion of infected roots, and by the absence of visible infection excepting when grown in wet soils particularly favorable for the disease. In the case of alfalfa and sweet clover, only young roots, before secondary thickening has disturbed the primary cortex, seem susceptible to attack. Very young alfalfa seedlings in diseased pea fields have been seen badly weakened and even killed by this disease, but such plants have constituted at most only a small fraction of the stand. In greenhouse trials, certain species of *Vicia* and

Lathyrus proved nearly as susceptible as peas, some of these seedlings being killed shortly after emergence. In general, species with coarse roots seem more susceptible than those with very fine roots.

The present list of hosts is such as to suggest that *Aphanomyces euteiches*, given particularly favorable conditions, may live as a weak parasite in the roots of a wide range of plants, not restricted to the Leguminosae. This supports the hypothesis that it may occur naturally in some soils prior to the first growth of peas, but as yet the rootrot fungus has not been found under virgin conditions in the roots of native plants. Rootrot has been observed several times in Wisconsin in recently cleared ground where peas were planted for the first time. The remarkably long persistence of the parasite in soils once thoroughly infested may now be readily understood. This wide range of hosts makes it apparent that rotation of crops with whatever interval between crops of peas cannot be confidently relied upon to eradicate the rootrot fungus from infested soil, even though experience has shown a long rotation to be a valuable aid in reducing the severity of infestation.

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FRANK J. PIEMEISEL

PHYTOPATHOLOGY

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FRANK J. PIEMEISEL

1891-1925

F. J. SCHNEIDERHAN

In the death of Frank J. Piemeisel the phytopathological world lost a young man whose talents gave every indication of future leadership in his chosen work. He was born at Jordan, Minnesota, June 30, 1891, and died November 24, 1925, at St. Cloud, Minn.

His elementary training was given him in the common schools at New Prague, Minnesota, from which he entered high school at Jordan, Minnesota, graduating in 1909. Before entering college he taught one term in a country school. In September, 1911, he entered the freshman class in the Forestry School of the University of Minnesota but at the end of this year changed to the Agricultural College. He was working his way through college and found work as assistant in the greenhouse laboratory courses in horticulture and agricultural botany. In his junior year he decided to make plant pathology his life work. He graduated from the University in 1914 with the degree of Bachelor of Science in Agriculture. His record during his undergraduate days was an excellent one, and he became a marked man for research work in plant pathology. He was honored at graduation by the award of the Shevlin Fellowship, which gave him the sum of \$600 and free tuition in the Graduate School, which he entered in the fall of 1914. In June, 1915, he obtained the M.S. degree. He was then employed by the Office of Cereal Investigations, Bureau of Plant Industry, where his research work stamped him as an outstanding investigator. He had made arrangements to pursue graduate work for his doctorate when he was drafted into the army on September 20, 1917, and was sent to France with the Meteorological Section of the Signal Corps. Piemeisel was in the first group of select men who went to war from his native town of Jordan. After serving 13 months overseas, he returned to the United States in February, 1919, utterly incapacitated for any type of work: the blight of

war left its tragic imprint on him, both physically and mentally. He received medical treatment at his home in Jordan and at Rochester, finally being sent to the Veterans' Bureau Hospital at St. Cloud, Minnesota, where he died of tuberculosis. Special memorial services were conducted in the auditorium of the Jordan High School, with speakers from the Minnesota State headquarters department of the American Legion. His funeral was a military one; the pall bearers were men who had served with him overseas.

Frank Piemeisel was a man of retiring disposition, and of the finest type of mentality. Even as an undergraduate he was far above the average of his classmates. He was endowed with a splendid memory and, although practically all of his studies were in biology, he possessed mathematical ability of a high order. He was a serious-minded student in whom the desire and joy of study was inborn. Never a pampered individual in boyhood, he grew up as a versatile, self-reliant student and scientific investigator whose untimely death was to his classmates and scientific associates one of the war's saddest casualties.

He was a fellow of the American Association for the Advancement of Science, a member of Sigma Xi and of the honorary agricultural fraternity of Alpha Zeta.

PUBLICATIONS OF FRANK J. PIEMEISEL

1914

Some facts of the life history of *Ustilago zeae* (Beckm.) Unger (Abs.) Phytopath. 4: 411.

1916

Infection of timothy by *Puccinia graminis avenae*. (With E. C. Stakman). Jour. Agr. Res. 6: 813-816.

1917

A new strain of *Puccinia graminis* (Abs.) (With E. C. Stakman). Phytopath. 7: 73.

Factors affecting the parasitism of *Ustilago zeae*. Phytopath. 7: 294-307.

Biologic forms of *Puccinia graminis* on wild grasses and cereals. (With E. C. Stakman). Jour. Agr. Res. 10: 429-496. (Prelim. report) Phytopath 6: 99-100.

1918

Can biologic forms of stem rust on wheat change rapidly enough to interfere with breeding for rust resistance. (With E. C. Stakman and J. H. Parker). Jour. Agr. Res. 14: 111-123.

Plasticity of biologic forms of *Puccinia graminis*. (With E. C. Stakman and M. N. Levine). Jour. Agr. Res. 15: 221-249.

THE PROBLEM OF *DICHROSTACHYS NUTANS*, A WEED TREE, IN CUBA WITH REMARKS ON ITS PATHOLOGY

1

JAMES R. WEIR

INTRODUCTION

In 1919 the writer's attention was called to the growing menace of the weed tree, *Dichrostachys nutans* (Pers.) Benth., on the arable lands in Cuba. At that time a report was prepared on certain fungi found on dead branches of the tree submitted for examination by Dr. Emilio D. Cassi, of Havana. This material was collected in a "Marabusal" of some 40 caballerias (about 1,350 acres) in which a disease was said to exist. Dr. Cassi in his letter described the disease as follows: "The bark at the base of the trunk turns a dull black, detaching itself from the tissues of the Marabon, while its roots and rootlets become fully rotten. I would state that the blight begins in the top of the tree and gradually descends to the roots." From what the writer has recently determined in the field the above statements are significant.

The following is quoted from the writer's original report: "Three common tropical fungi were found on the specimens submitted and are responsible for the decay in the wood. They are *Polystictus pinsitus*, *Trametes rigida* and *Corticium portentosum*. These fungi are not parasitic and are secondary to and follow the true causal agent of the disease." Nothing more was learned on the subject until the writer's visit to Cuba under the direction of the Tropical Plant Research Foundation in November, 1924.

THE TREE

Dichrostachys nutans, or marabu, is a small tree belonging to the Leguminosae indigenous to Africa and Asia. It is said to have been introduced in Cuba from Senegal as an ornamental. The tree has the habit of some Acacias and Mimosas and has been referred at different times to *Mimosa*, *Desmanthus* and *Cailliea*. The last, according to Macbride (2), is the first generic name used for the plant. The name for the tree is then *Cailliea glomerata* (Forsk.) Macbride. Regarding this name change, Macbride states that, notwithstanding Forskal's meagre characterization of his plant, it is obvious that he was naming the species later known as *Dichrostachys nutans*, since this is the only member of the Mimosidae growing in Arabia which has "Folia bipinnata" and "Legumen nigrum controto-globosum."

The resemblance of the tree to species of native *Acacia*, in particular to



FIG. 1. Road through dense impenetrable growth of marabu. Area previously under cultivation.

Acacia farnesiana (aromo amarillo), has led to the belief in Cuba that more than one species of *Dichrostachys* exists. Likewise the common name of *Acacia farnesiana* is often applied to the marabu. In many parts of Santa Clara province the common name of aromo or aroma was used exclusively. Roig (4) has pointed out the characters of the two species.

STATUS OF THE PROBLEM

Although the importance of marabu as a pest was learned from correspondence, it was a matter of surprise to find that it was even more serious in many localities than was anticipated. In the provinces of Matanza, Santa Clara, and Havana, the tree is abundant. It occurs in Pinar del Rio and was observed in Camaguey and Oriente. The tree is especially abundant in Santa Clara province. Brother Leon, in a letter in 1919, very aptly described the situation, and the writer's observations and photographs verify his remarks. "The tree grows so dense that the branches meeting at 5 or 6 meters above the ground cause the roads and paths cut through

to appear as tunnels (Fig. 1). The dense verdure sometimes extends for hundreds of meters uninterruptedly. From a neighboring hill it appears somewhat like a lawn or like Bermuda grass, and, sparkling as it does with handsome flowers, forms one of the most beautiful features of the Cuban Flora. In the Trinidad Valley and in the region surrounding Cienfuegos the tree is unchecked and forms veritable forests on hill land or on areas on which cane growing has been discontinued (Fig. 3). In the early stages of the formation of these forests the tree forms thickets quite impenetrable on account of density and the abundance of the long, stiff, sharp thorns (Fig. 3). Later, with increasing size of the trees, the natural pruning of the lower branches and the dying-out of the suppressed individuals, it is possible to walk over the area without much difficulty. In the fully mature stands where the trees have attained diameters from 4-6 inches the lower branches have fallen away in some cases so that it is even possible to ride through the thickets on horseback, but trails are necessary for easy progress.

Mr. H. A. van Herman (5) calls attention to the pest, stating that "whole farms in central Cuba have been rendered useless by this foreign nuisance without any effort being made to check the curse and that good farm land is being abandoned in disgust."

Whenever cane land fails, from one cause or another, to produce the required tonnage, it is frequently allowed to run wild or revert to pasture. On such areas, owing to the treatment the soil has received, the marabu finds optimum conditions for growth. Small patches of the tree make their appearance here and there over the area. From these centers the tree spreads in all directions by means of the advancing horizontal root system and by means of the seeds. The distribution of the tree is probably effected in part by cattle carrying seeds in their hoofs when driven or transported over the island. Cattle also greatly relish the seeds, and it is possible that they are capable of germination after passing through the animals.

Marabu has a well developed tap root which in good soil penetrates to a considerable depth. Lateral horizontal roots extend unusual distances in all directions. In addition there are supporting roots at an acute angle to the tap root and frequently many fibrous roots. The lateral or horizontal roots may lie at varying depths but are usually only a few inches below the surface of the ground. They may be pulled up for a distance of several feet. It is these roots that make eradication difficult. If the parent tree is cut down, these roots at once sprout profusely. If their removal is attempted, any small section left in the soil immediately develops fibrous roots and produces shoots. It was observed on areas where the parent trees were felled that the prolific development of young plants from the lateral roots

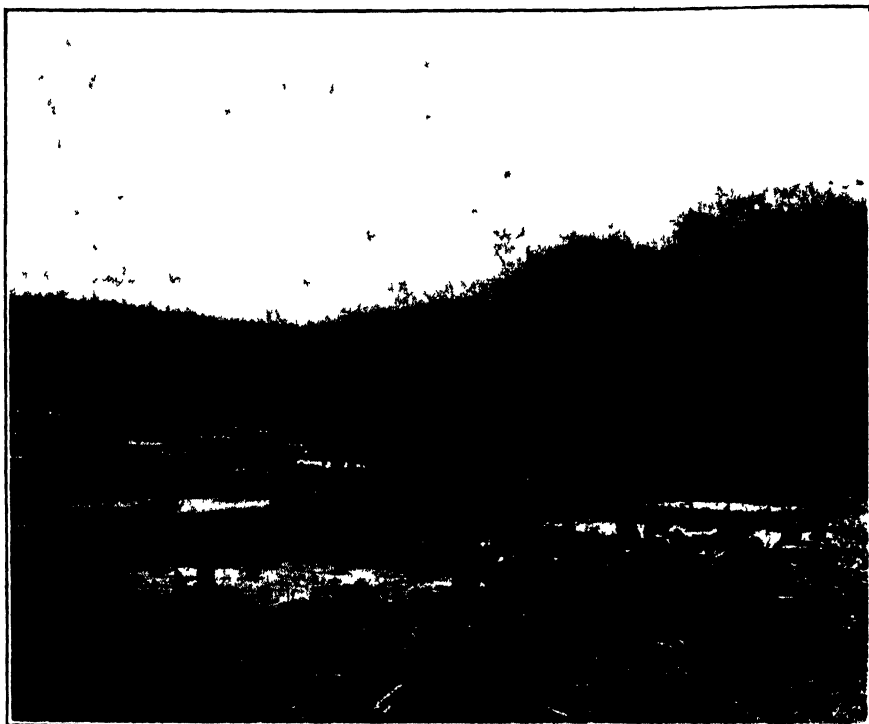


FIG. 2. Forest of marabu being cleared by slashing and burning Old tree in the foreground apparently killed by *Ustilina zonata*

was greatly stimulated. The ground on such areas soon becomes carpeted with small plants, and the whole on examination will be found to spring from the wide-flung root system of the parent tree. Root grafting is common between different individuals in a dense stand. It is easy to imagine that there may well be a common root system for the stand as a whole.

ERADICATION

Individual plants may be effectively killed by the application of crude petroleum to the roots. It is said that this treatment causes the death of the entire root system and that the odor of the petroleum may be detected in the roots at their farthest extremities. This is somewhat contrary to the usual expectation and should be thoroughly tested. The petroleum should not be too thick, otherwise it will not penetrate either the soil or the roots. Salt, kerosene, and sodium-arsenite have been used effectively in barberry eradication and would no doubt be equally effective in killing marabu. The last, however, is extremely poisonous and care should be taken in its use. Individual trees also may be killed by girdling with a gasoline blow torch. The writer (6) has used this method of killing trees

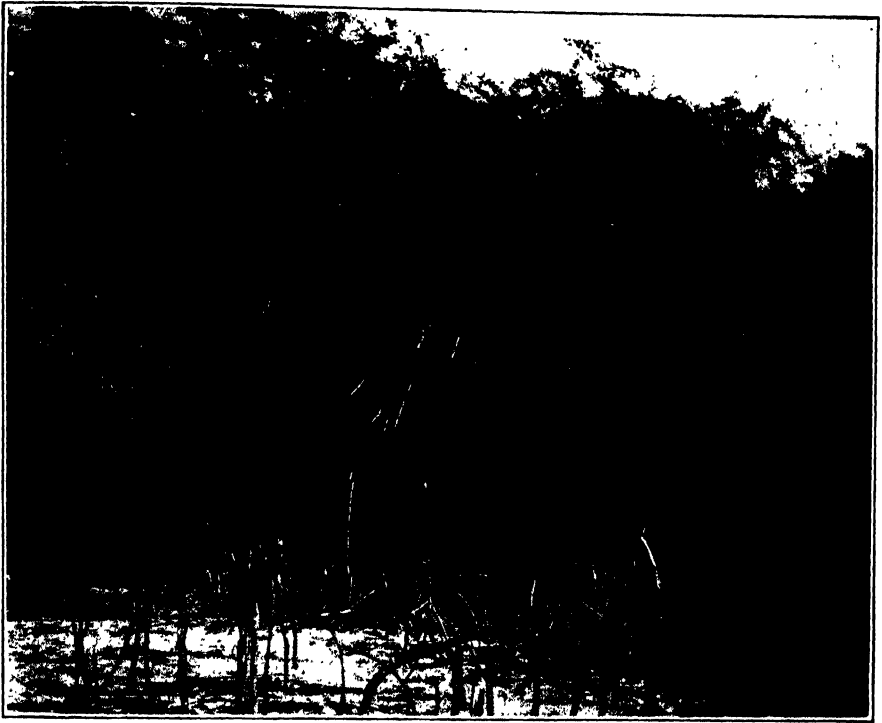


FIG. 3. Dense growth of marabu on previously cultivated land, showing the dense crown canopy and young sprouts.

in the North and found it to be effective and time-saving. A torch may be devised by which a small tank may be carried on the back and connected with the burner by means of a hose and short length of rigid tubing.

After the tree is once established in pure stands the only practical means known at present of eradication over large areas is by slashing and burning (Fig. 2). This is done at considerable expense, and only a high profit crop such as sugar cane will pay for the effort. The broadcast burning of the area, however, does not destroy the root system. The ground is soon carpeted with young root sprouts. If cane is planted on the area, frequent hoeing is necessary until the cane is big enough to shade the ground. If the area is maintained continuously in cane for a protracted period and the sprouts are prevented from developing, the area may be effectively freed from the pest. Some varieties of cane are more effective in shading the ground than others. Uba cane, owing to its density, is said to prevent the growth of marabu more than any other variety.

Although it is not known to what extent the foliage of the marabu is eaten by stock, still it would appear that the young plants on a newly cleared area may be kept down by grazing goats if the land is not immediately util-

ized. After the plants have become woody and covered with thorns it is not likely that they would be eaten.

The bark and wood of the marabu contains tannin. The setting up of plants for the extraction of tannic acid may be a worthwhile consideration. In this connection, comparison is made with *Acacia decurrens*, *A. mollissima* and *A. dealbata*, the tannin-producing wattles of South Africa and Madagascar. The wood of marabu makes an excellent charcoal and is much used for this purpose. It would appear that the commercial utilization of the tree might be undertaken at a profit.

While in Cuba the writer heard of a cattle man who instructed his field staff to immediately destroy all marabu plants encountered. This resulted in his estate being free from the pest, although surrounded by a dense growth of the tree.

DISEASES OF MARABU

No further notice of a disease of marabu, after that mentioned in a letter by Dr. Cassi, came to the writer's attention until the spring of 1924. At this time Dr. W. H. Weston sent to the writer a fungus which he had collected on marabu at the central Trinidad. The fungus (*Ganoderma pulverulentum*) was found in the root crotches and also attached to the horizontal roots of dead and dying trees at the central Trinidad. Later the writer examined the same area and collected the fungus in abundance (Pl. VI, A). A close examination of the affected trees leads to the opinion that this fungus is able to attack the trees only under certain conditions, such as the presence of fire scars, wounds made by the tramping of cattle or by bark and wood-boring insects. Entering through wounds, the fungus attacks the heart wood, causing a whitish undifferentiated decay. This decay is in striking contrast with the sound, dark brown heart wood. The mycelium may follow in or along decaying horizontal roots and develop fructifications some distance from the base of the tree.

Ganoderma pulverulentum does not produce white mycelial layers in or beneath the bark. The white fan-like structure often found in association with it belongs to the fungus described here. The fructifications are of the usual *Ganoderma* type of the stipitate group and need not be described. The species has been described several times as new, but the name *Ganoderma pulverulentum* Murr. may be used for the present. *Polyporus opacus* Berk. and Mont., a species characterized by a light-colored zonate pileus and very rough spores, is also found on the roots of marabu. The species is rare and apparently of no importance in Cuba.

A close examination of the trees on the area where *Ganoderma pulverulentum* was found disclosed the interesting fact that the primary cause of the death of the trees was *Ustulina zonata* (Lev.) Sacc. This fungus is widely distributed in the tropics and is known to attack a large number of hosts. It is chiefly known as a cause of a root and trunk disease of coffee,

cacao, tea, rubber, and other perennial crops. The writer has recently described the fungus in detail in a report on the diseases of *Hevea* in the Amazon Valley, and only a brief account of its characteristics is necessary here.

The marabu trees attacked by this fungus begin to die gradually. The leaves turn yellow and fall. Even before the tree dies the fructifications of the fungus appear around the base or on the lateral roots (Pl. VI, B, Pl. VIII, A and B). These first appear as small pustules which enlarge, become confluent with similar structures and eventually form broad white or grayish white plates of several inches in extent (Fig. 4). During this stage conidia are produced as free spores on the surface of the plate (Pl. VII, B). The growth of the plates may be arrested by change in weather conditions resulting in concentric zonations of the surface (Fig. 4). If growth is continuous the disks may be smooth except when growing over irregular surfaces. After the procutio production of the conidia, at which time the plate is soft, it becomes hard and changes color to grayish green, then purple-black and eventually becomes quite black, hard and brittle. The surface is then covered with small black dots (Pl. VII, F). These are the openings to the perithecia (Pl. VII, E) which produce the ascospores or second spore form (Pl. VII, D). The nature and appearance of these structures are well shown in plate VII and in figure 4.

Ustulina zonata is considered to be identical with *U. vulgaris* Tul. of the temperate zone by some authors and by reasons of priority should be referred to *U. maxima* (Weber) v. Wettst. For comparison the appearance of the average spores of the two forms are shown in Pl. VII, A, B, C and D. It would appear that the material here studied does not belong to one species.

Ustulina zonata does not form an external vegetative mycelium. If the bark is removed, a white fan-like mycelium is disclosed (Pl. VI, C, and Pl. VIII, C), which covers the surface of the wood as a continuous sheet. It is this mycelium which girdles the tree or its roots and destroys the cambium, resulting in the death of the tree. The wood is permeated with black lines, as is usually the case with fungi of this group (Pl. VIII, D). The wood of young roots may be evenly blackened by the development of the mycelium in the vessels (Pl. VIII, E). Before the tree dies or afterwards, wood-destroying fungi appear, resulting in the decay of the roots and stems.

Ustulina zonata was found at several stations in the Trinidad Valley. Only at Central Trinidad was it associated with the *Ganoderma*. The fungus was invariably found at the bases of dead trees and those with yellow foliage.

The observations show that *Ustulina zonata* attacks and kills the marabu. This being a gradual process, the use of the fungus in eradication work will be determined by future investigations. A series of inoculations have been



FIG. 4. *Ustilina zonata* on marabu, showing the varying form and appearance of the encrusting fructifications.

made in the field, the results of which will be reported later. Since the parasite in Trinidad Valley was especially associated with the marabu, it seems likely that we are dealing here with a special strain of the fungus which may not attack the more permanent crops of the region.

It is believed that a full and careful study of the marabu situation will result in devising a practical and rapid means of disposing of the pest. To this end more should be known of the diseases and pests of the tree in the countries where it is indigenous. A leaf-eating insect which has been determined by Dr. A. G. Böving, of the Bureau of Entomology, as *Tenebriodes mauritanicus* (L.) was found to defoliate the tree in some regions in Cuba. The same insect, however, also was found on *Acacia farnesiana*.

A large number of species of fungi were collected on dead and dying marabu but, with the exception of the two described, all are saprophytic.

DANGER OF INTRODUCTION INTO THE UNITED STATES

The danger of introduction of the tree into the United States is very great. There seems to be little doubt that it would find suitable conditions in the southern and southwestern parts of the country. Bailey (1) has reported its introduction into southern California. Reed (3) is of the opinion that the shrub would work great damage to the cattle ranges in California if once introduced. It would be worth while to investigate rumors that the plant has been introduced into Florida.

SUMMARY

1. *Dichrostachys nulans* (Pers.) Benth., or marabu, a dangerous weed tree, was introduced into Cuba from Senegal, and has become a serious menace on the arable lands. It forms veritable forests on abandoned cane land where there are optimum conditions for its growth.

2. Eradication is extremely difficult because of the prolific sprouting of the lateral roots after the parent tree has been cut.

3. Individual plants are effectively killed by the application of crude petroleum, or other substances used in the eradication of barberry.

4. The only known practical method of eradication over large areas of pure stands is by slashing and burning.

5. The commercial utilization of the bark and wood of the marabu for tannin and charcoal products is a possibility worthy of consideration.

6. Two parasitic fungi, in addition to various saprophytic forms, are found associated with dead and dying trees. *Ganoderma pulverulentum* Murr. enters through wounds, causing a white, undifferentiated decay of the heartwood.

7. The primary cause of the death of the trees, however, is *Ustulina zonata* (Lev.) Sacc., a fungus with a wide host range in the tropics. The

production of white, fan-like mycelium beneath the bark of the tree is characteristic of it.

8. An exhaustive and careful study of the diseases and pests of the marabu should result in the discovery of a practical means of disposing of the tree.

9. Conditions are favorable for the growth of this pest in the United States, and the danger of introduction is great.

OFFICE OF MYCOLOGICAL COLLECTIONS AND DISEASE SURVEY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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DESCRIPTION OF PLATES

PLATE VI

- A. *Ganoderma pulverulentum* growing from scars at the base of marabu.
- B. Fructifications of *Ustulina zonata* at the base of living marabu.
- C. Bark removed from a living marabu tree showing the advance of the white mycelial layer of *Ustulina zonata* between the bark and the wood.

PLATE VII

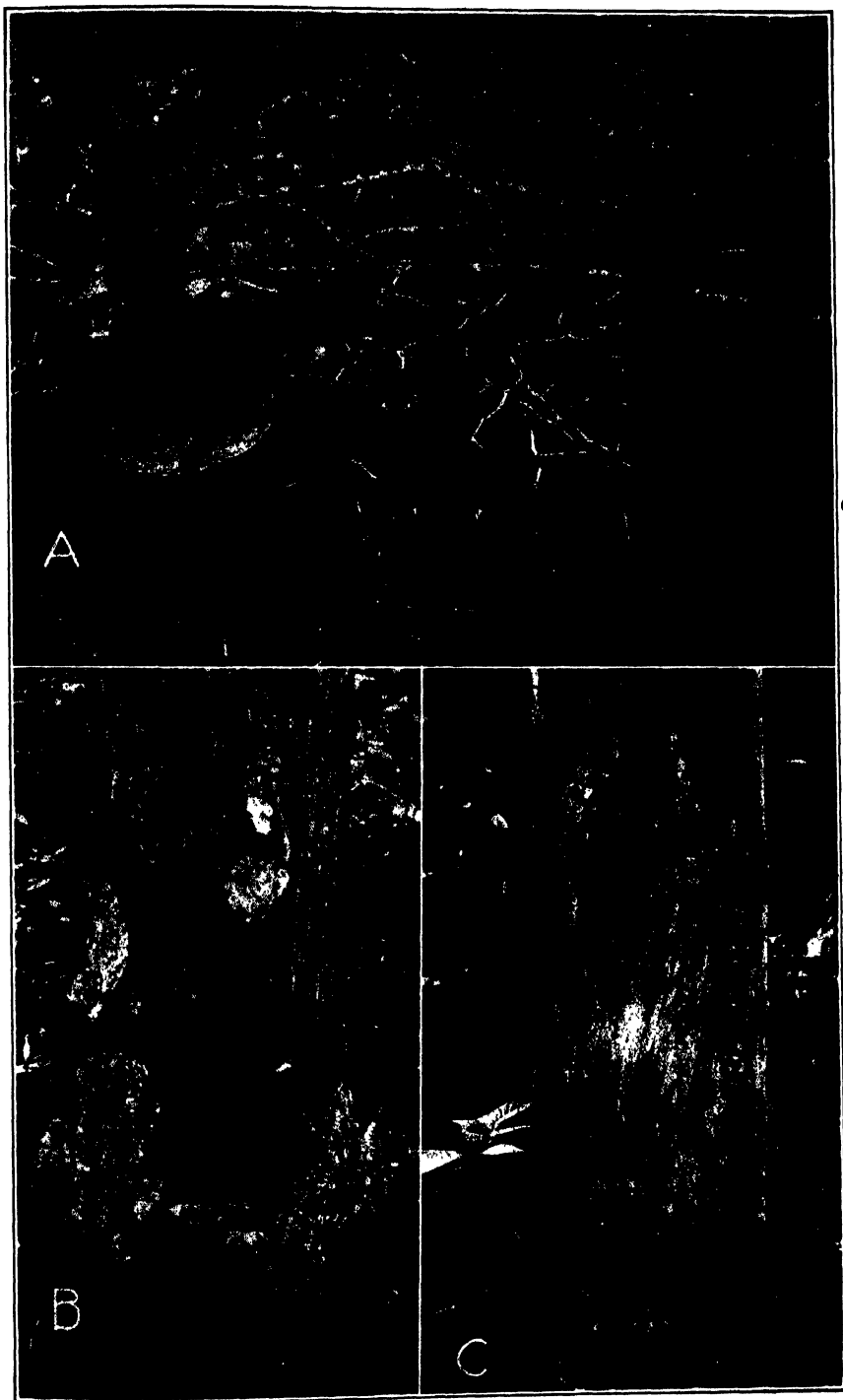
Contrast of *Ustulina zonata* and *U. vulgaris* of the temperate zone.

- A. Conidia of *U. vulgaris* $\times 400$.
- B. Conidia of *U. zonata* $\times 400$.
- C. Ascospores of *U. vulgaris* $\times 400$.
- D. Ascospores of *U. zonata* $\times 400$.
- E. Perithecia of *U. zonata* enlarged (rule in 16ths of an inch).
- F. Openings of same surface of fructification, enlarged (rule in 16ths of an inch).

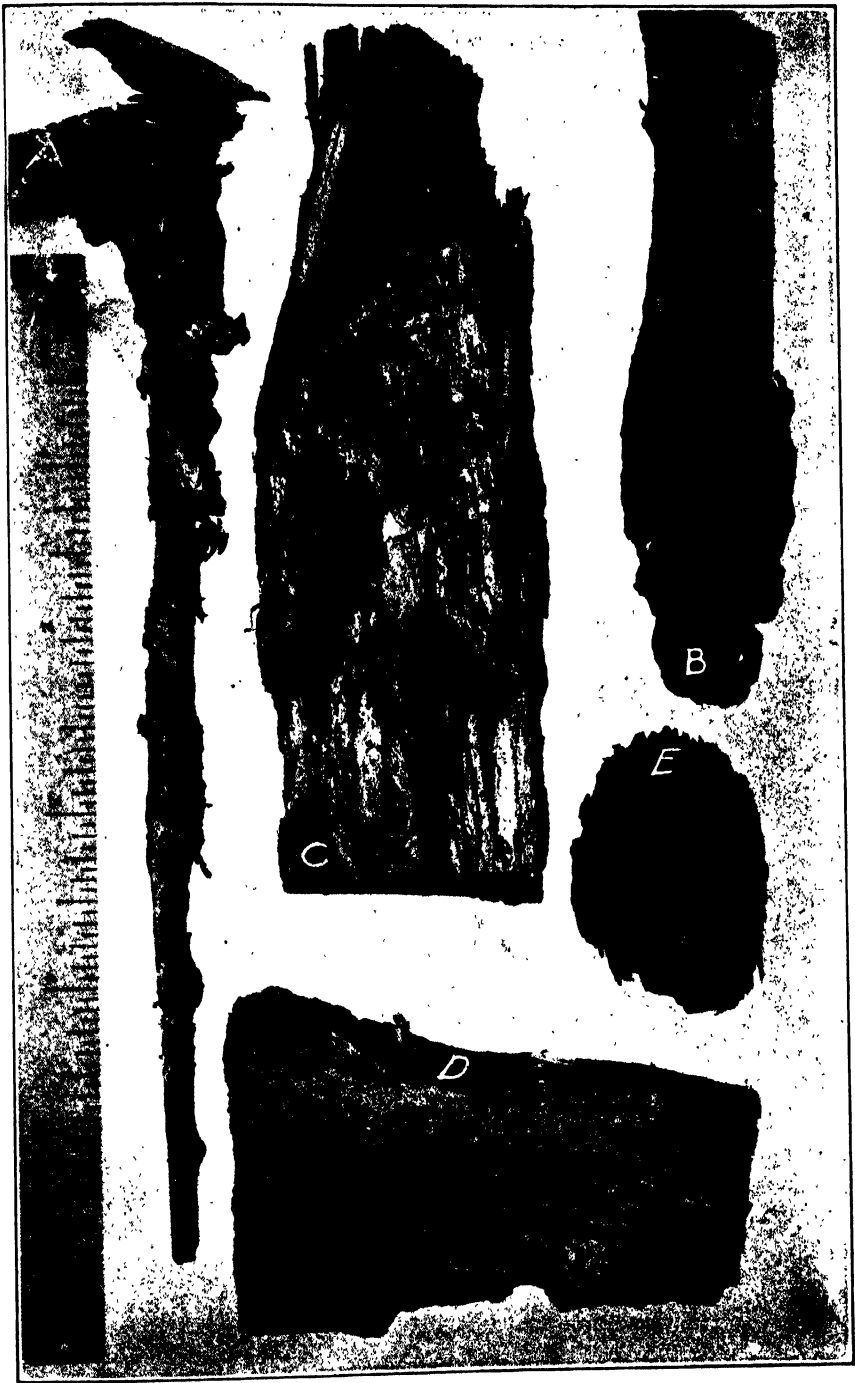
PLATE VIII

Ustulina zonata on marabu.

- A. On small lateral root.
- B. At the base of a small tree.
- C. Mycelial fans in the zone of the cambium.
- D. Black lines in the wood.
- E. General discoloration of the wood without formation of black lines.







PLANT DISEASE NOTES FROM THE CENTRAL ANDES

CARLOS E. CHARDÓN AND RAFAEL A. TORO

At the invitation of the Colombian Government, the senior author spent two months—from April 16 to June 15, 1926—in the city of Medellin, organizing the agricultural department for the State of Antioquia. Since all travelling facilities were placed at his disposal, there was ample opportunity to take notes on the plant diseases of that region. The plant diseases of northern South America are very imperfectly known, owing to a lack of interest on the part of the governments in agricultural science. Thus the plant diseases of a large country as Colombia, whose area exceeds that of France and Germany combined, are wholly unknown, except for the work of the Swiss naturalists Fuhrmann and Mayor (5). The latter author, who is essentially a mycologist, gives a fairly complete account of the rust flora, and incidentally presents a fair discussion of various coffee diseases. Aside from this work, nothing is known in that remote country about plant diseases. As an illustration of this ignorance, it may be stated that as late as 1910 a serious outbreak of the *Stilbum* leaf spot in Cundinamarca was mistaken for the coffee rust, and great alarm was caused. Fortunately, at that time the Swiss expeditioners reached Bogotá, and Dr. Mayor promptly decided that there was nothing serious. Thus it has been deemed advisable for the present authors to write a few notes on the diseases observed.

The portion of the country visited is only a small area of the national territory. Properly speaking, it is a portion of the Central Andes. The great Andean Cordillera, as it progresses northward toward the Isthmus of Panama, becomes subdivided into three ranges: the Western Andes which are limited by the Pacific Ocean and the Cauca River; the Central Andes, which are limited by the Cauca and Magdalena Rivers, including the lovely Medellin valley; and the Eastern Andes, which run east of the Magdalena River, cover the fertile Bogotá plateau, extend to Venezuela and terminate in the snowy peaks of Sierra Nevada, in the Santa Marta peninsula. The Bogotá plateau, where the capital is located, is an extensive and rich plain, 8,000 feet above sea level. Wheat, oats, all kinds of temperate grains and fruits thrive well there. This area was not covered by the senior author, who restricted himself to the State of Antioquia, or more definitely, to the Medellin valley, including the Central Andes to the Cauca valley. (See Fig. 1.)

The valley of Medellin is 25 miles long and 4 to 5 miles wide. It is 4,500 to 4,800 feet above sea level and has a climate of perpetual spring. Medel-

lin, the capital of the State of Antioquia, is a very progressive town of over 80,000 inhabitants. It is a great coffee center, the home of the well known "Medellin Excelso" coffee, which commands the highest price in the New York market. The soil of the Medellin valley is generally poor: it has become exhausted through continuous use. Cane, corn, and beans (frisoles) are grown and there is good pasture land. South of the valley, from the town of Caldas, the country becomes rugged, and mountains ranging from 5,000 to 7,000 feet cover the territory as far as the Cauca River. These mountains are mainly of volcanic origin, the soil decomposing into a porous, gravelly loam rich in organic matter. The mean temperature ranges from 59° F. at 6,000 to 6,500 feet to 64.5° F. at 5,500 to 6,000 feet. Conditions for coffee growing are ideal here; yields are high and the berry is of extra quality. Curiously enough, sugar-cane is always grown in all the big coffee "haciendas," being cultivated in the lower lands of the plantations. Sugar is manufactured by primitive methods and a very crude brown sugar (panela) is produced. Corn and beans are universally cultivated and constitute two very important articles of food. A few temperate fruits are cultivated in the backyards of many country residences, but the apples and pears examined were quite small; peaches (duraznos) seemed to be the best adapted of the temperate fruits. The delicious "cherimoya" (*Anona cherimoya*), the queen of the tropical fruits, reigns supreme in the higher lands.

The following plant diseases were found to be prevalent by the senior author, during his relatively short visit:

Sugar-Cane "Gomosis."

A general infection of gomosis was encountered in many fields of sugar cane. The leaf symptoms were invariably those described by Matz (7) from Porto Rico and frequently observed by the senior author in the infected cane fields of that island. Longitudinal streaks of dead tissue were found beginning near the leaf tip and extending well into the leaf, running parallel to the rib. The gummy exudation of the stalk, when cut, however, was not so characteristic. Very few diseased stalks examined showed the yellow gum oozing from the fibrovascular bundles in the cut surface. This was apparently due to the very prolonged drought that prevailed in Antioquia during March, April and May. Similar cases of infected canes showing no exudation after a long, dry spell are of frequent occurrence in Porto Rico.

Microscopic examination of the diseased stalks showed the vessels plugged with the yellow gum. Disintegration of the wall of the vessels was also observed, as in typical cases of cane gomosis.

The cane variety most universally planted in Antioquia is a clean-looking yellowish cane with long internodes. It is known as "Castilla" cane,

and is claimed to have been introduced many years ago, when it completely replaced the old, degenerate "Criolla." The senior author believes this Castilla variety is the same as the "Bourbon" or "Otaheite" cane of the West Indies. In Antioquia, the Castilla variety has been grown for many years on poor soils so that it shows signs of degeneration yields, 8 to 10 tons

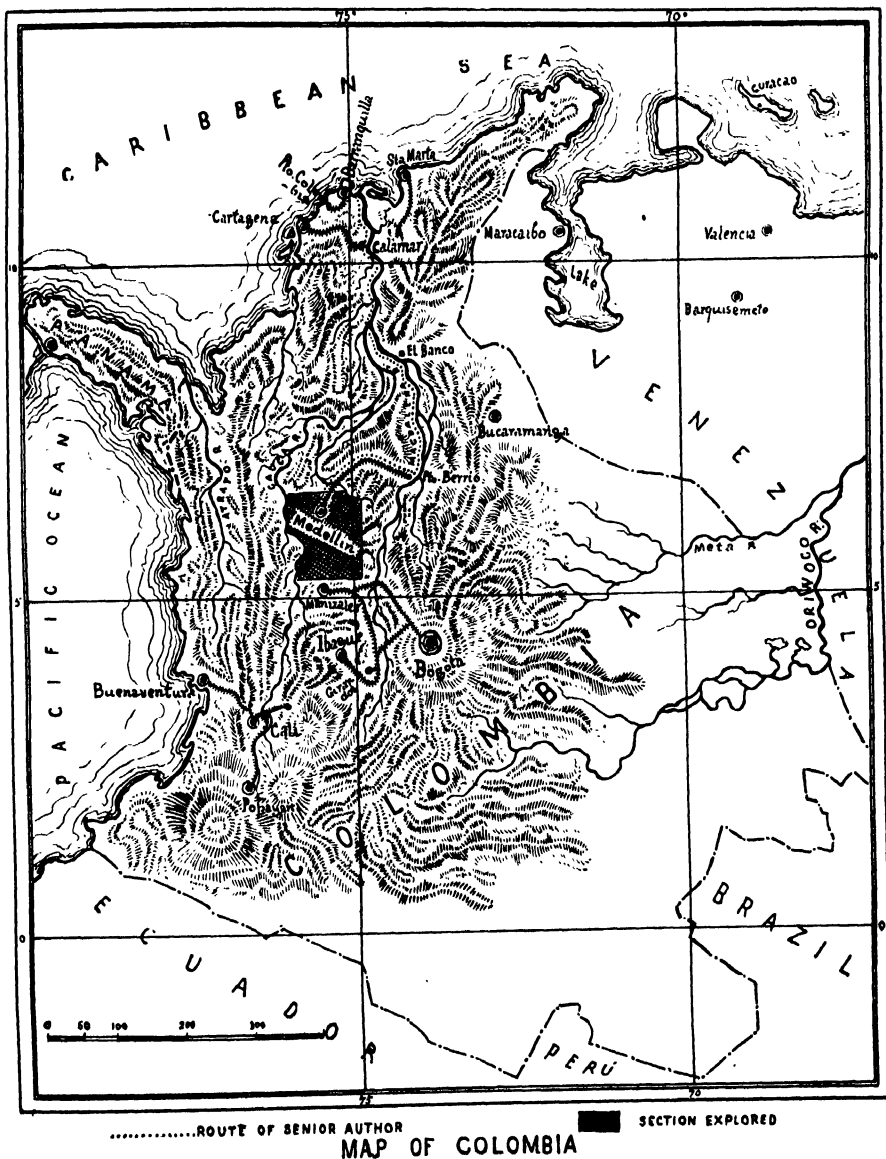


FIG. 1. Map of Colombia showing the route of the senior author and the area surveyed. Scale in kilometers.

being the average. Some "Cavangerie" and "Cristalina" canes are also grown in limited quantities. It is probably safe to assume that 90 per cent is Bourbon or Otaheite cane.

Since this appears to be the variety most susceptible to gomosis, the seriousness of the situation is very apparent. Many fields were completely infected, diseased seed was used everywhere, and there were great losses in the mills. The manager of ingenio "Santana" reported "great difficulties in the vacuum pans due to an unknown cause." The disease is so destructive that a popular circular on gomosis was issued and promptly distributed among all farmers (1).

It was impossible to trace when and how the cane gomosis found its way to the Central Andes. The disease is not known in Cuba or Santo Domingo; but in Porto Rico it was first reported by Matz (6) in 1921. No exchange of seed is known to have occurred between the latter island and any of the Colombian ports. Furthermore, the disease has been established in the Central Andes for many years, probably prior to its appearance in Porto Rico. The disease was probably introduced from Brazil, where cane gomosis had been reported by Dränert (2) as a serious malady as early as 1869.

A complete displacement of the susceptible Otaheite cane with more tolerant and higher sucrose yielding varieties is most imperative. The B.H.10(12), which is being so widely used in the British West Indies and in Porto Rico, is a cane very resistant to gomosis. This excellent variety, together with the S.C.12/4 and some of the early maturing P. O. J. canes, ought to be given a trial, and their importation to Antioquia has been strongly recommended to the Government.

Coffee Root Disease.

The etiology of the root diseases of coffee is still obscure. There are two apparently distinct coffee root troubles in America. One, reported by d'Herelle (3) from Guatemala in 1909, is known as "amarillamiento" (yellowing). He claims that it is caused by a new Pyrenomycete, which he names *Phthora vastatrix*. The fungus occurs as black bodies underneath the bark, near the base of the trunk. These black bodies are the stromata which enclose a single row of tightly pressed perithecia. The name "amarillamiento" has been applied because of the yellowing of the leaves of the diseased trees, which later dry and fall to the ground about the time of the tree's death.

Mayor (8) reported the amarillamiento as occurring in the State of Cundinamarca, Colombia, in 1912. He visited the coffee plantations of the Viola district, examined and collected plenty of diseased material, which he sent to Paris for comparison, and the disease was diagnosed as the amarillamiento of d'Herelle.

The other coffee root disease is the *Rosellinia* root disease of the West Indies. This malady is thoroughly described by Fawcett (4) from Porto Rico in 1915. It is said to be caused by the fungus *Rosellinia bunodes*; but perithecia were reported in two cases only, one of them being on a species of *Piper*. Specimens of the perithecial stage of the fungus have not been found at the Mayaguez Experiment Station. Thus the question of the perfect stage of the *Rosellinia* causing root disease of coffee in Porto Rico is not entirely proved. The conidial stage of the fungus (*Dematophora* sp.), however, is very common.

On the author's arrival at Medellin, repeated questions were asked him as to the presence there of a coffee root disease which was called the "llaga." It was reported to kill coffee trees in the Fredonia district. Repeated attempts to find the disease failed: many coffee trees were found dead, but their death was attributable to either white grubs or trunk borers. Finally the so-called llaga was found at the "Amparo" coffee plantation. The disease was not common, probably owing to the prolonged dry season, but the few trees examined showed unmistakable symptoms of root disease.

A few dried leaves were found on the branches of the trees which showed that their death had been recent. No external symptoms on the trunk above the surface of the soil were observed, but large black areas were seen on the roots just below the solid surface. These black areas were the llaga (canker). The cortex of the diseased root had already gone, exposing portions of the wood. The exposed wood was all black, and a hand-lens examination showed the presence of very minute fungous threads. Under the microscope they look like the *Dematophora* reported and pictured by Fawcett (4) as occurring on coffee root disease in Porto Rico. No *Rosellinia* stage was found. Careful search for *Phthora vastatrix* d'Herelle was also unsuccessful.

Careful comparison of the symptoms of the llaga found in Antioquia with those described by d'Herelle from Guatemala and Fawcett from Porto Rico show both diseases to have similar external symptoms. A careful investigation on the etiology of these coffee root troubles, however, needs to be made before definite relations are to be established between the root diseases found in various countries.

Coffee Leaf Spot.

The widely known leaf spot, caused by *Omphalia flavida* Maubl. et Rang., was not found on account of the drought, but it was stated to be common in shady and well protected places during the rainy season. It is known as "gotera." The disease has never been reported severe in the State of Antioquia. Years ago, it was very severe in the State of Cudinamarca, where it was mistaken for the coffee rust disease. So far, coffee rust is unknown to America.

Sooty Mold of Coffee.

Very severe cases of sooty mold were examined. The fungus (*Capnodium brasiliense* Pat. and Maubl.) completely covers the surface of the leaves, the branches and even the berries. There is little doubt that the fungus injures the coffee trees by interfering with production. The sooty mold was found to be associated with an abundant cottony scale. However, the sooty mold is in itself more unsightly than harmful and is important chiefly in indicating the presence of the scale insects with which it is associated. The fungus is dependent on the honey dew secreted by these insects and disappears when they are destroyed.

Tobacco Mosaic.

Mosaic is quite prevalent on tobacco, and a high percentage of infection is often found in the fields. No one seems to care about it, nor even notice it, but the leaves showed evident damage and distortion.

Mosaic of Grasses (Cane, Corn, etc.).

Diligent search for mosaic was made on practically all the sugar plantations, but the senior author failed to find the mosaic on sugar-cane. The disease has not found its way up the Magdalena River yet. Corn did not seem to be infected either. The corn plantings were in good condition, and the corn aphid (*Aphis maidis*) was not found. The whole State of Antioquia may be safeguarded against this dangerous disease through the strict observance of proper quarantine regulations.

Bean Rust (Uromyces appendiculatus).

Rust is quite prevalent on the common bean, lima beans and the native "frisoles," but the damage observed was not important.

Fig Rust (Kuehneola fici).

Various fig trees with severe infections of rust were examined. The leaves were distorted and yellowish, showing evident damage from the rust.

Rind Disease of Sugar-Cane.

Although the rind disease of sugar cane, caused by the fungus *Melanconium sacchari* Massee, is considered by some as a serious parasite, most workers are now agreed that it is but a wound parasite and only attacks weakened or over-matured canes. The prevalence of this fungus in Antioquia can be easily explained, as has already been said, by the deteriorated varieties and very poor soil.

Ring Spot of Sugar-Cane.

Ring spot is a leaf disease of sugar-cane caused by the fungus *Leptosphaeria sacchari* van Breda de Haan. Two other species of *Leptosphaeria* on cane have been described from South America by Spegazzini (9). One of them is also called *L. sacchari* Speg. It does not appear to be very severe although it causes considerable discoloration of the leaf, especially at the tips.

Citrus Scab.

Scab was observed on some branches and leaves of the sour orange, *Citrus aurantium*. Some cases of distortion of leaves were seen and also falling of distorted leaves. The causal organism is a fungus recently described as *Sphaceloma fawcetti* Jenkins.

Other minor diseases.

Coconut leaves were found attacked by the fungus *Pestalozzia palmanum* Cooke; tomato leaves were heavily infected by the fungus *Septoria lycopersici* Speg; and *Phyllachora gratissima* causes a tan spot on avocado leaves.

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THE NATURE OF SEED-PIECE TRANSMISSION OF POTATO BLACKLEG¹

J. G. LEACH

Since the early investigations of Appel (1) on potato blackleg, it has been generally assumed that infected seed-pieces constitute the chief means of dissemination and hibernation of the blackleg pathogene. In fact, many investigators considered this to be the sole source of infection (6, 7). Although the statement is frequently made that "the organism . . . is carried over the seed tuber" (2), or that "infection results from the planting of diseased seed or possibly seed that has come in contact with diseased seed" (4), very little has been said about the histological details of such infected tubers. Morse (5), in 1909, states "the evidence thus far obtained indicates that blackleg is largely distributed by means of germs carried in wounds, cracks, and decayed areas of seed pieces." In 1917 (6) he again states his views as follows: "It is the writer's opinion that they are carried over winter

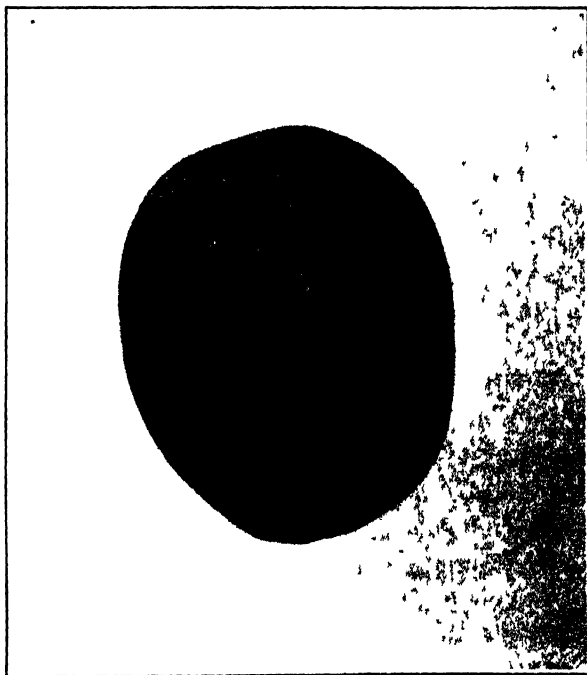


FIG. 1. A potato tuber taken from a plant affected with blackleg showing typical stem end infection.

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FIG. 2. A potato tuber similar to the one shown in figure 1, cut longitudinally through the point of attachment of the stolon, showing how the discolored area tends to follow the vascular bundles.

in decaying, cracked, or otherwise imperfect tubers . . . therefore it seems probable, if the bacteria are able to enter the interior tissues of the tuber either by natural infection in the field before harvesting, or through lesions produced by other parasitic organisms that, so long as they are supplied with a small amount of moisture, they will remain alive. The low temperatures of storage prevent their rapid multiplication and the resultant decay of the tubers. It is undoubtedly those only slightly affected tubers which are responsible for the propagation of the disease."

Orton (8) considered that contamination of the freshly cut surface of seed-pieces with bacteria from the cutting knives was sufficient to produce infection of the resulting plants and recommended disinfection of the cutting knives as a precaution. Other workers have expressed similar conceptions of seed-piece infection.

The writer (3) recently reported experiments in which a large number of seed-pieces, partly decayed with the blackleg pathogene, were planted under various soil conditions without producing the disease. The efficiency with which the seed-piece corked off the decay was pointed out and it was concluded that "the results indicate that the contamination of the seed-piece at the time of cutting usually is not an important factor in the dissemination of the pathogene."

Although there is a great deal of observational evidence that seed tubers taken from fields having a high percentage of blackleg tend to produce more blackleg than seed from disease-free fields, a survey of the literature reveals

no experiments in which tubers from diseased plants had been selected, planted, and observed in order to determine the frequency of the occurrence of diseased plants as compared with the progeny of tubers from healthy plants.

In the late summer of 1924, a quantity of tubers were selected from plants affected with blackleg. Only those tubers were saved which showed more or less decay at the stem end, which had spread into the tuber from the decayed stolon (Figs. 1, 2, and 3). The following January, 100 of these tubers were cut in half longitudinally and planted in 8-inch pots in the greenhouse. An equal number of tubers from healthy plants were inoculated with the blackleg pathogene according to the method previously described (3). When the decay was well advanced, the tubers were cut in half, planted in 8-inch pots, and placed on the bench beside the other pots in a cool corner of the greenhouse. The pots were kept well watered until maturity. On account of the low temperature, the tubers sprouted slowly and two of the artificially inoculated tubers completely decayed before forming a sprout. As the season advanced, the average temperature of the green-

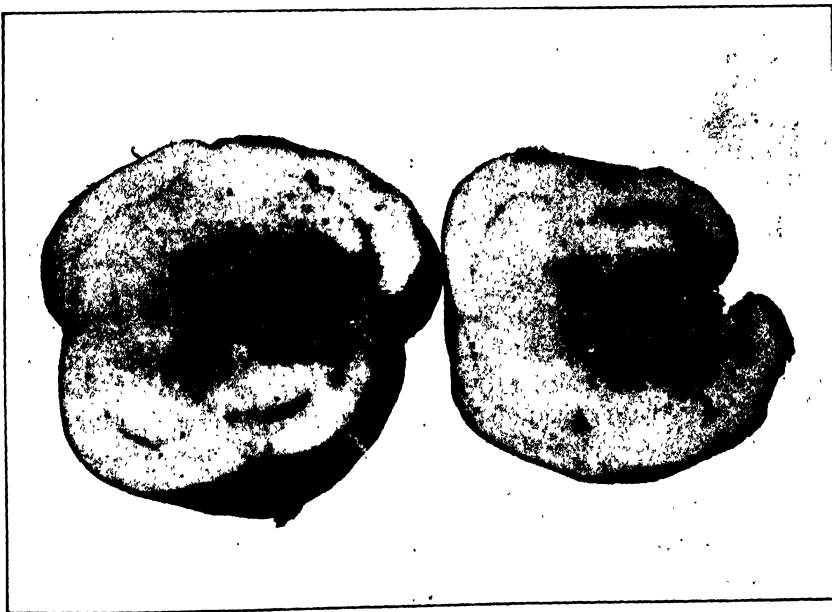


FIG. 3. A potato tuber showing a more severe case of stem-end infection. Although a considerable amount of parenchyma had decayed before the tuber was dug, when the tuber was examined in January a thick layer of cork had formed which apparently had checked the decay. However, the vascular bundles were destroyed for some distance beyond the wall of cork and, when planted, each half of the tuber produced a blackleg plant.

house rose and the plants grew at a normal rate. Twenty-four plants from the naturally infected tubers developed unmistakable blackleg, while only one plant of the artificially inoculated series developed the disease. The disease did not manifest itself until the plants were well advanced toward maturity. Tuber formation was well under way.

In 1925, another lot of naturally infected tubers were collected. These were kept in cold storage until the following spring when they were planted in the field. An equal number of artificially inoculated tubers were planted in adjacent rows. In this experiment, nine per cent of the naturally infected tubers produced blackleg plants, whereas none of those artificially inoculated produced diseased plants. Here also the disease did not appear until the plants were well advanced.

Seed-pieces, dug up when the first symptoms of the disease appeared, nearly always were found to have been affected with a watery, translucent decay. The slimy decay frequently associated with the seed-pieces of diseased plants was rarely found. In many cases the seed-pieces retained their original shape and structure, the covering of cork appearing to be intact.

The results of these experiments would seem to indicate that the natural inoculation of tubers through stem-end infection from diseased plants is much more effective than artificial inoculation. With the view of finding an explanation for this fact, a histological study was made of naturally infected tubers before planting and during the early stages of the development of the disease.

The results of this study may be summarized briefly as follows: When a stem-end infected tuber is cut in half longitudinally through the point of attachment of the stolon, it readily may be seen that the decay extends farthest along the vascular bundles (Figs. 2 and 3). The presence of the pathogene in such lesions has been demonstrated by isolations in which it has been obtained in a fair percentage of trials. Whenever any considerable amount of the parenchyma has been destroyed, careful examination shows that the decay has been checked and cut off from the rest of the parenchyma by a layer of cork (Fig. 3). It appears that it would have been entirely cut off except for its ability to invade the vascular bundles. The cells of the vascular bundles, being unable to form cork, could not stop the advance of the bacteria. As soon as the bacteria advance beyond the cork layer, formed by the parenchyma cells, they again break out of the vascular elements into the surrounding parenchyma but are soon checked by a layer of cork which quickly forms around the vascular bundle. Figure 4 shows a photomicrograph of a longitudinal section of an infected vascular bundle around which has been laid down a thick wall of cork cells. Thus the bacteria slowly advance down the vascular elements where they obtain sufficient moisture to remain alive, but from which they are unable to escape



FIG. 4. A photograph of a section through a vascular bundle from a stem-end infected tuber, such as the one shown in figure 2. The elements of the vascular bundles have been destroyed, but the spread of the bacteria was checked by the parenchyma, which formed a cylinder of cork cells around the old bundle. As soon as the starch has been removed from the seed-piece by the sprout, the parenchyma is no longer able to form cork and the bacteria spread unhindered throughout the seed-piece and into the growing sprout.

on account of the effective cork wall laid down by the parenchyma cells surrounding the bundles.

When such tubers are planted, the bacteria are unable to escape from the vascular bundles until the starch has been removed by the sprout. When this stage is reached, the seed-piece is no longer able to form cork, so the bacteria develop rapidly throughout the seed-piece and spread into the stem producing the disease.

In order to prove the validity of the assumption that the seed-piece is unable to cork off the decay after the starch has been removed, a number of sound seed-pieces were removed from growing plants and inoculated with the blackleg pathogene. Normal tubers were inoculated at the same time, and all were placed over water in a closed chamber through which a stream of air saturated with moisture was drawn by means of a suction pump. The seed-pieces from which the starch had been removed were completely decayed within three days while the inoculated normal tubers remained sound and completely corked off the decay.

This study leads to the conclusion that blackleg may be systemic in nature and may be perpetuated by tubers naturally infected through the vascular bundles which enter the tuber from decaying stolons. Artificial inoculation through the parenchyma tissues appears to be relatively ineffective in producing the disease unless aided by some agency which inhibits cork formation.

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UNIFORMITY OF NOMENCLATURE FOR THE VIROSES OF SOLANUM TUBEROSUM

DONALD FOLSON

Variation and overlapping of the symptoms of bacterial and fungous diseases, with the existing differences in national languages, render descriptive or so-called common names unsuitable as universal or standard names of such diseases. However, with respect to these diseases we can cite the pathogene for purposes of universal terminology, so that the standardization of terms can be approximated in spite of difficulties that have been encountered in the proving of pathogenicity and in bacteriological and mycological nomenclature (7, 8, 15, 16).

Certain aggregates of symptoms in potatoes (*Solanum tuberosum*) are considered to be due to corresponding degeneration diseases or viroses.¹ The existing evidence shows that one virosis may have different aggregates of symptoms as the result of differences in (1) the variety of potato, (2) the stage of growth of the potato plant, (3) the recency of infection, (4) the prevailing environmental factors, and (5) the number and kinds of other viroses affecting the plant (13, 14). Not only does the same virosis have different symptom aggregates, but, with a close relationship between some causal agencies apparently existing, the same symptom aggregate sometimes is ascribable to several viroses. That is, there is an overlapping of symptoms (12). This situation is like that with respect to bacterial and fungous diseases. However, there is no relief through referring to a pathogene. Of the viroses, the "aetiology will continue to be a subject of discussion until successful and convincing inoculation experiments with pure cultures of . . . possible organisms have established their relation to any of these diseases" (11, p. 24); or, it may be added for the benefit of those inclining to some other theory of causation, until the causal agency, of whatever type it may be, is isolated, identified, and proved to be the causal agency.

In the absence of knowledge of a causal agency that can serve as a tolerable universal name at least among scientists, it has been advocated that attempts be made to standardize the descriptive names of potato viroses. In standardizing in this way, the selected name will have a double use—as a more or less local descriptive name and also as a universal standard name that may not be descriptive in many localities. Such an attempted distinction may not be practicable, and at least will be confusing.

¹ A name for "virus diseases" proposed on December 28, 1925, at Lincoln, Nebraska, by Dr. L. R. Jones.

It has been maintained also, on the other hand, that it is futile to try to standardize the names of viroses in any way until the causal agencies have been determined. The inference to be drawn from this is that the determination of the causal agencies will greatly facilitate standardization. However, if we are to believe some authorities, such determination will only be the beginning of our real troubles, at least if the priority principle is followed (7, 8, 15, 16). At any rate, an indefinite postponement of a solution by deciding to wait until the causal agencies are discovered will not prove satisfactory to those workers desiring standard terms to be available soon.

There are two worthy motives for such standardization in potato viroses: the scientific and the practical. The scientific motive is a desire to attain to a more satisfactory analysis of the objective problem. The practical motive is a desire to expedite control of the diseases through certification, agricultural extension instruction, and other means that require a knowledge of names.

Among phanerogamic taxonomists the tendency seems to be toward standardization through the agreement of an authoritative, representative committee upon a definite list of standard names, following the lead of a group of practical men (1). "If these names are to be adopted by all practical and professional horticulturists, florists, nurserymen, and pharmacists, as is indicated by the endorsement of their national organizations, the professional taxonomists will find little use [on the part of the practical men] for any new or old names which they attempt to substitute or reinstate for those in this list" (15).

It is possible that students of a group of diseases can, or should, make further progress toward the standardization of terms than the students of mycology or of botanical taxonomy as a whole (3).

The preceding three paragraphs may suggest some leading of action by practical men with regard to potato viroses. However, it is quite possible for each state to have its own certification requirements, terms, and interpretation of terms and thus to bring about a local standardization that is satisfactory. That is, in practice it results in the best seed producible in that region. Also, seed growers can detect and eliminate virotic plants (those affected with viroses) without having been instructed as to the names of the viroses present. At any rate, there is no immediate prospect of the formation of a practical committee which will decide what synonyms deserve exclusive use in trade, as has been done with respect to the horticultural plants (1). Possibly the practical men realize the practical difficulties more keenly than do some who advocate immediate standardization.

Some scientific workers think it possible both to attain to a more satisfactory analysis of the objective problem and to advance standardization of terms through a comparison of published descriptions. For example, after

the statement that "mosaic itself is now believed to comprise several distinct forms of disease," the symptoms of mosaic are given as mottling and crinkling; and subsequently there is given: "Crinkle.—This disease appears to be the same as the rugose mosaic of Schultz and Folsom in the United States of America." (2, p. 45, 48, 51.) Also, "Streak.—There is probably more than one form of streak. The form described by Orton and later by Atanasoff has been called by the latter stipple streak." (2, p. 51.)

Atanasoff, discussing the question of uniformity of names, is more pessimistic. He states that:

"Words like 'dwarfing,' 'wrinkling,' . . . 'curly-dwarf' . . . etc., . . . are meaningless because they are too broad and too general and because they are not sufficient in themselves to describe a whole complex of symptoms. What is still worse is that one author calls a certain disease 'leaf roll,' another author speaks of the same disease as 'leaf curl'; one uses the name 'streak,' the other the name 'stipple-streak' for the same disease; one speaks of 'crinkle,' the other of 'rugose mosaic' and they both mean the same disease. . . . The only result of this deplorable practice is that even the men working on this problem cannot understand each other's writings, let alone those who are not familiar with these diseases." (4, p. 521–522.)

The preceding suggests the question, is it consistent to state that names are meaningless when two such names are said to belong to the same disease? Also, is it consistent to apply a new name to what is believed to be the same disease and then to deplore the bad example thus set? There may rather be a need for a retention of names already in use, when possible; for definitions of terms applied to unit symptoms (14, p. 44–45); and for a desire to understand such terms rather than to try to become famous through initiating new ones. However, there is a difficulty still more fundamental than those which have been referred to. Even with all workers on potato virores cooperating as much as is possible through the literature and correspondence, a general agreement as to the facts will come about only with a liberal exchange of carefully grown tubers perpetuating different diseases. Even with that practice established, there will have to be liberality in regard to uncontrollable mistakes following transmission by as yet unknown agencies, and comparisons should be made as far as possible in controlled environments (5, 6, 9, 10, 17).

Some of the quotations given in previous paragraphs indicate incidentally the best present method of designating a potato virosis, that is, to name the virosis, the author of the original description, and if possible the date and place of publication. After the materials upon which such descriptions are based have been exchanged and studied in a truly cooperative spirit, eventually it may be possible to assemble a committee with authority and knowledge enough to select synonyms which will be regarded as standard. Such

a committee probably will have to include one or more representatives of the chief phytopathological society of each nation, or, if there is no such society, then the chief botanical society, with at least a four-fifths majority required to list a name as approved or as synonymous with an approved name.

Perhaps there is need for such a committee to consider viroses of all plants instead of potato viroses alone. In such a case each species of plant should have a list of viroses authorized, and the identity of viroses of different plants should be shown in a suitable manner. Possibly it would be simpler, and as satisfactory on the whole, to designate the different viroses of a species by Arabic numerals. We might thus have designated, for example,—“*Solanum tuberosum* L., virosis 1. Described as *rugose mosaic* by E. S. Schultz and Donald Folsom in Jour. Agr. Res. 25: 52–53, 61. 1923. Synonym *crinkle* as described by Paul A. Murphy in Canada Exp. Farms Div. Bot. Bul. 44, II. 71–74. 1921. Identical with *Nicotiana tabacum* L., virosis 5, and with *Lycopersicon esculentum* Mill. virosis 2.” If, in spite of many data and due deliberation, the committee should allot a number to a combination of viroses, when the mistake becomes evident it would be easy to state that the so-called virosis is a combination of certain new ones. A numerical designation as a standard would be simple, would be indefinitely expansive, would be impartial to the different theories of causation and relationship of viroses, and would be usable by all writers employing Latin binomials of the host plants. It could, in fact, be used without the names of the host species in a single series of numbers.

It would be a desirable step for an authoritative committee at least to agree upon what is required in the way of proof that a virosis has been sufficiently studied to deserve a standard name.

SUMMARY

Reasons and methods have been advanced for the standardization of names of potato viroses. The writer suggests an authorized scientific committee for the selection of satisfactory descriptions and their standard designation by number in a separate series for each species of host plant, or in a single series.

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A STUDY OF THE DISTRIBUTION OF *TILLETIA TRITICI* AND *T. LAEVIS* IN 1926¹

W. H. TISDALE, C. E. LEIGHTY, AND E. G. BOERNER

During the past few years the losses in the United States due to stinking smut of wheat have been increasing very noticeably. Estimated reductions in yield during the years 1917 to 1924 indicate that the losses run from 5,000,000 to 26,000,000 bushels a year. The average estimated reduction in yield during this period was over 14,000,000 bushels annually. The greatest loss occurred in 1924, when over 26,000,000 bushels were destroyed by stinking smut. This wide range in the amount of reduction in yield, with the heaviest loss occurring in 1924, may be attributed to the fact that an intensive campaign for smut control was conducted by the United States Department of Agriculture in 1918 and 1919. This resulted in a greatly increased use of seed treatment which, no doubt, was responsible to a large extent for the low percentages of smut in the earlier years of this period. After this campaign had become forgotten there was a decrease in the amount of seed treatment due probably to a large extent to the rapid decline in the price of wheat about 1920, which caused the farmers to lose much of their interest in the crop. The result was a gradual increase in the percentage of smut infection which culminated in the enormous loss in 1924. In addition to this reduction in yield there is a very heavy loss due to price discounts on smutted wheat at the market. These discounts range from a few cents to 25 cents per bushel.

The prevalence of smut in 1924 is largely responsible for the subsequent heavy losses in 1925 and 1926 in the Middle West and the Eastern States. This was due to the use of smut-infested seed wheat in those years. In the regions named, soil infestation is not an important factor and the smut is spread principally with the seed. In the Pacific Coast States, where soil infestation is an important factor, epidemics of smut cause a heavier infestation of both seed and soil.

There has been considerable speculation regarding the cause or causes of this increase in stinking smut, especially east of the Rocky Mountains. Weather conditions following sowing of wheat are known to influence infection. Temperatures ranging between 5° and 15° C. are highly favorable

¹ Investigations conducted cooperatively between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, and the Grain Division of the Bureau of Agricultural Economics, U. S. Department of Agriculture.

for infection by either species of *Tilletia*.² Some of the state pathologists are inclined to believe that the increase in the percentage of smut is due to the more general practice of sowing late to escape Hessian fly injury. The late sowing, of course, subjects the germinating seed to lower temperatures, which in turn favor infection. As previously stated, one of the chief causes for the increasing percentages of smut may be the decrease in seed treatment resulting from the low smut infection following the smut control campaign in 1918 and 1919. This may be one of the chief reasons for the increases of stinking smut. Of course, it is necessary that the seed carry smut spores if infection is to take place, as soil infestation is seldom known to cause infection east of the Rocky Mountains. Once the smut occurs in a locality it is easily spread through the distribution of seed and by the use of the threshing ring where the same machine is used on several farms.

Even though the later sowing of wheat seemed to favor increased smut infection, the writers developed another theory. They thought it possible that *T. tritici*, the species commonly present in the Pacific Coast region, and known to occur locally east of the Rocky Mountains, might have become widely distributed in the East and for some reason might be more virulent than *T. laevis* under eastern conditions, and thus be responsible for the smut epidemics. A report by Haskell in The Plant Disease Bulletin, June 20, 1919, on the distribution of the two species of *Tilletia*, showed the occurrence of *T. tritici*, the western species, only as far east as Indiana and Michigan. *T. laevis* occurred from coast to coast but was found to be much more prevalent east of the Rocky Mountains. In this survey, however, very few collections of smut were made in the States east of the Mississippi River. It seemed possible that *T. tritici* might have become spread throughout the East since that time. There also is the possibility of strains of different virulence, in either one or both of these species, which might account for the increasing prevalence of the disease. With these points in mind, 900 addressed seed envelopes were sent to nine grain terminals east of the Rocky Mountains, 100 to each terminal, with a request for samples of smutted wheat from receipts from various localities within their respective territories. About 560 samples were received, each representing a carload shipment from a country point, and the species of *Tilletia* present were determined. Tables 1 and 2 and the accompanying map give the distribution of the two species of *Tilletia* as shown by these samples.

A study of the tables and the map (Fig. 1) do not indicate that *T. tritici*, the western species, is at all responsible for the epidemics of stinking smut east of the Rocky Mountains.

² Faris, J. A. Factors influencing the infection of wheat by *Tilletia tritici* and *Tilletia laevis*. Mycologia 16: 259-282. 1924.

TABLE 1.—*Geographic distribution of Tilletia tritici and T. laevis in the four states, Minnesota, Montana, North Dakota and South Dakota, where both were found, as indicated by their occurrence in samples of smutted wheat originating at the points named, in 1926*

Where grown		Terminal	No. of samples	Species of <i>Tilletia</i>
State	Locality			
Minnesota	Barnesville	Duluth, Minn.	1	<i>tritici</i>
	Dumont		1	do
	Kenyon		1	do
	Tenney		1	<i>laevis</i>
	Billings	Minneapolis, Minn.	1	do
	Brushvale		2	do
	Buffington		1	<i>tritici</i>
	Hallock		1	<i>laevis</i>
	Lafayette		1	do
	Maplelake		1	do
	Seaforth		1	do
Montana	Benchland	Duluth, Minn.	1	do
	Brampton		1	<i>tritici</i>
	Bush Siding		2	<i>laevis</i>
	Charlo		1	do
	Hysham		1	do
	Laurel		1	do
	Libby		1	do
	Springfield		1	do
	Big Sandy	Minneapolis, Minn.	1	do
	Columbus		1	do
	Denton		1	do
	Glentana		1	do
	Hobson		1	do
	Mocassin		1	do
	Poplar		1	do
	Underdahl		2	do
North Dakota	Absaraka	Duluth, Minn.	1	<i>laevis</i> and <i>tritici</i>
	Adams		1	<i>tritici</i>
	Ahame		1	<i>laevis</i> and <i>tritici</i>
	Alice		1	<i>tritici</i>
	Bremen		1	do
	Buffalo		1	do
	Casselton		1	do
	Casselton		1	<i>laevis</i>
	Cheyenne		1	<i>tritici</i>
	Churchs Ferry		1	do
	Clements ville		2	do
	Amherst		1	do
	Buttzville		1	do
	Thompson		1	do

TABLE 1.—*Continued*

Where grown		Terminal	No. of samples	Species of <i>Tilletia</i>
State	Locality			
North Dakota	Dahlen	Duluth, Minn.	1	<i>tritici</i>
	Dalrymple		1	do
	Delamere		1	<i>laevis</i> and <i>tritici</i>
	Egeland		1	<i>tritici</i>
	Elizabeth		1	<i>laevis</i>
	Enderlin		2	<i>tritici</i>
	Erie		1	do
	Florence		1	do
	Frazier		1	do
	Glasston		1	do
	Golden Valley		1	do
	Grandin		1	<i>laevis</i>
	Hannaford		1	<i>tritici</i>
	Hensol		1	<i>laevis</i>
	Jessie		2	<i>tritici</i>
	Josephin		1	do
	Lamars		1	<i>laevis</i>
	Lanona		1	do
	Lawton		1	<i>tritici</i>
	Lead		1	do
	Ledgerwood		1	do
	Maza		2	do
	Maxbass		2	do
	Minnewaukin		1	do
	Munster		1	do
	Munster		1	<i>laevis</i> and <i>tritici</i>
	Mylo		1	<i>tritici</i>
	Northwood		2	do
	Odessa		1	<i>laevis</i>
	Osnabrock		1	<i>tritici</i>
	Ottertail		1	<i>laevis</i>
	Parduloc		1	<i>tritici</i>
	Persis		1	<i>laevis</i>
	Petersburg		1	<i>tritici</i>
	Rauville		1	do
	Reeder		1	<i>laevis</i>
	Rogers		1	<i>tritici</i>
	Stirum		3	do
	St. Thomas		1	<i>laevis</i>
	Venango		1	do
	Walum		1	<i>tritici</i>
	Webster		1	do
	Wheaton		1	do
	Wishek		1	<i>laevis</i>
	Ypsilanti		1	<i>tritici</i>

The tables and map show that *T. tritici*, the western species, was found only in samples grown in Montana, North Dakota, South Dakota, and Minnesota. These samples were received from Minneapolis and Duluth. Of the 71 samples in which only *T. tritici* was found, all were durum wheat. Many of these, however, contained small admixtures of common wheat. Seven of

TABLE 1.—*Continued*

Where grown		Terminal	No. of samples	Species of <i>Tilletia</i>
State	Locality			
North Dakota	Baden	Minneapolis, Minn.	1	<i>laevis</i>
	Blanchard		1	do
	Brandt		1	<i>tritici</i>
	Clements ville		1	<i>laevis</i>
	Corinth		1	do
	Forman		1	do
	Hillsboro		2	do
	Lansford		1	do
	Marshall		1	do
	McClusky		1	do
	Mott		1	do
	Parshall		2	do
	Tunbridge		1	do
	Warwick		1	do
	Wimbledon		1	<i>laevis</i> and <i>tritici</i>
South Dakota	Blackwell	Oklahoma City, Okla.	1	<i>tritici</i>
	Bristol	Duluth, Minn.	1	do
			1	<i>laevis</i>
			1	<i>tritici</i>
			1	do
			1	do
	Belvidere	Minneapolis, Minn.	1	do
	Creston		1	<i>laevis</i>
	Draper		1	<i>tritici</i>
	Faulkton		1	<i>laevis</i>
	Freeman		1	<i>tritici</i>
	Garden City		1	do
	Grover		1	do
	Hart		1	<i>laevis</i> and <i>tritici</i>
	Kampeska		1	<i>tritici</i>
	Labolt		1	<i>laevis</i>
	Langford		1	<i>tritici</i>
	Lemmon		1	<i>laevis</i>
	Oelrichs		1	do
	Peever		1	<i>tritici</i>
	Philip		1	<i>laevis</i>
	Strandburg		1	<i>tritici</i>
	Unity Ville		1	do
	Wessington Springs		1	<i>laevis</i>
	Hitchcock		1	do

these 71 samples were principally red-kerneled durum, three were mixtures of red-kerneled and amber-kerneled durums, and the remainder were principally amber-kerneled durums. The five samples from North Dakota and the single sample from South Dakota, in which both *T. laevis* and *T. tritici* were found, were mixtures of durum and common wheats, the approximate percentages of durum being 94, 80, 88, 90, 3, and 96 per cent, respectively.

In all of these samples very few smut balls were present, and only two samples contained kernels with smutted tips, and but a few of these. It is possible that *T. tritici* was produced on the common wheat in some or all of these samples, but *T. tritici* was not found in the samples of common wheat in which no durum was present.

If the samples examined can be considered as representative, they furnish strong indications that *T. tritici*, in the upper Mississippi and Missouri Valleys, is confined almost entirely, if not entirely, to durum wheat. It is not known why this should be the case, as all the common wheats grown in that region are more or less susceptible to infection by *T. tritici*, and the durum

TABLE 2.—Summary showing the occurrence of *Tilletia tritici* and *T. laevis* in samples of wheat collected at several grain terminals in the Middle Western and Eastern States in 1926

Regions and State or Province	No. of towns	Number of samples containing <i>Tilletia</i>		
		<i>tritici</i>	<i>laevis</i>	<i>tritici</i> and <i>laevis</i>
West of Mississippi River				
Northern Section				
Minnesota	11	4	8	
Montana	16	1	17	
North Dakota	58	52	28	5
South Dakota	24	14	9	1
Central and Southern Sections				
Colorado	14		17	
Kansas	60		79	
Nebraska	31		40	
Oklahoma	33		59	
Texas	6		15	
Utah	1		1	
East of Mississippi River				
Delaware	23		50	
Illinois	2		2	
Indiana	9		12	
Iowa	5		5	
Maryland	38		68	
Michigan	1		1	
Ohio	8		11	
Pennsylvania	33		46	
Virginia	2		3	
Canada				
Ontario	2		2	

wheats are likewise susceptible to infection by *T. laevis*. Both durum and common wheat are grown in the same localities, often on the same farm. They are sown with the same drills, handled with the same machinery and threshed in the same machines. Samples of common and durum wheats were received from the same general area and it is hardly likely that durum has not had opportunity for infection by *T. laevis* and the common wheats by *T. tritici*.

Another question regarding *T. tritici* is why it should remain confined to limited areas east of the Rocky Mountains, where there are so many possibilities for its spread. Perhaps there are climatic factors which limit its distribution. *T. laevis* was found throughout the entire area from which these samples were received, extending from Maryland and Delaware on the Atlantic Coast to Utah and Montana in the West.

Although it is not possible to determine strain differences within the species until further study is made, it is certain that, if a specially virulent strain is responsible for the epidemics east of the Rocky Mountains, it is a strain of *T. laevis*, the species commonly occurring in the East. In examining the samples considerable differences in the shape and appearance of

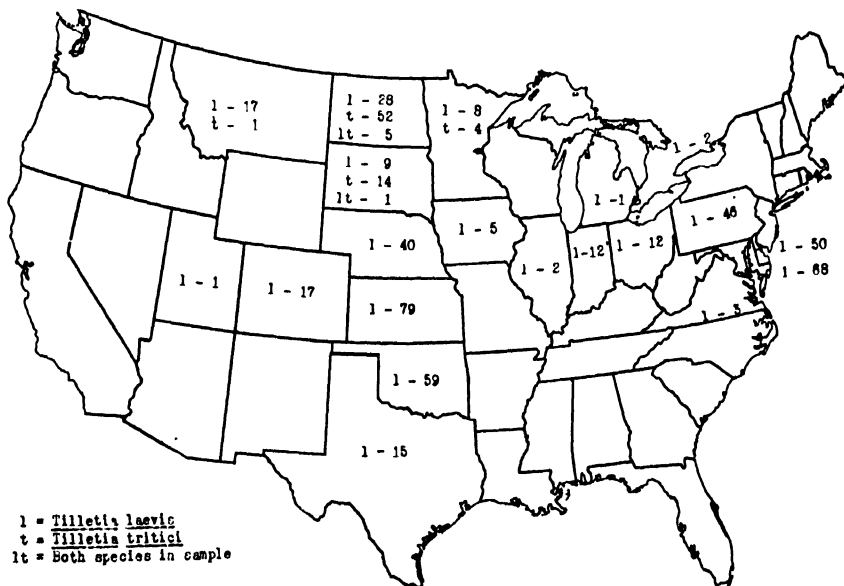


FIG. 1.—Map showing the distribution of *Tilletia tritici* and *T. laevis*, causing stinking smut of wheat, in a number of samples of wheat obtained from several marketing terminals in 1926.

spores of *T. laevis* were noted in different samples and at times in different smut balls within the same sample. The spores of *T. tritici* varied from very echinulate to almost smooth. In fact, the two species seemed to merge or overlap in this respect. This morphological variation may or may not be correlated with any physiological variations that may exist within the species.

OFFICE OF CEREAL CROPS AND DISEASES,

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE

EXPERIMENTS WITH DUSTS FOR CONTROLLING STRIPE DISEASE OF BARLEY¹

R. W. LEUKEL, JAMES G. DICKSON, AND
A. G. JOHNSON

The widespread use of copper carbonate for the control of bunt during the past few years naturally suggested the use of dusts for controlling other seed-borne diseases of cereals. Howitt and Stone (7) found that copper carbonate controlled smut in Liberty Hull-less oats. Heald, Zundel and Boyle (5) reported perfect control of oat smut with copper carbonate in Chinese Hull-less oats but not in the hulled varieties Swedish Select and Abundance. Sampson and Davies (11) found that copper carbonate reduced the amount of smut in hull-less oats 70 per cent. Dickson (1) also found nickel and copper dusts satisfactory for controlling smuts in hull-less oats. Martin (9) reduced the amount of smut in oats from 4.13 per cent to 0.22 and 0.85 per cent with nickel carbonate and copper carbonate, respectively. He did not state whether hulled or hull-less oats were used. Thomas (12) found that, although copper carbonate, nickel carbonate, and copper sulphate dusts used alone were ineffective against oat smuts, a mixture of any of these with mercuric chloride controlled the oat smuts satisfactorily.

Holbert, Reddy and Koehler (6) report increased yields after dusting *Diplodia*- and *Gibberella*-infected seed of dent corn with various organic mercury compounds. Reddy, Holbert and Erwin (10) likewise reported similar results from dusting *Diplodia*- and *Gibberella*-infected seed of sweet corn with certain organic mercury compounds. The increased yields doubtless indicate at least partial control of the seed-borne diseases mentioned.

Gram (4) reported some success in controlling stripe disease of barley by dusting the seed with powdered Germisan and Tillantin "C". Dorph-Petersen (2) stated that out of 12 barley fields, seed for which had been dusted with various new preparations, eight remained free from stripe disease, while the rest showed only a trace of infection. Gisevius and Straib (3) completely controlled stripe disease of barley with the two dusts, Höchst and Tutan, using them at the rate of 4 grams per kilogram of seed. Abavit "B" reduced the amount of stripe to 1.5 per cent, while the controls showed 5.4 per cent. In preliminary experiments in 1925 with a number of

¹ Investigations conducted cooperatively between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

dusts, the writers (8) obtained results in the control of stripe disease of barley which appeared to justify further work along this line. Accordingly, somewhat more extensive seed-treatment experiments with dusts for stripe-disease control were undertaken in the spring of 1926.

MATERIALS

Dusts which previously had been found ineffective (8) in controlling stripe disease were not included in these experiments. Following is a list of the dusts used in the experiments here described, together with the names of the commercial concerns manufacturing them.

1. Abavit "B."—Chemische Fabrik Ludwig Meyer, Mainz, Germany.
 2. S. F. A. No. 225
 3. S. F. A. No. 225V
 4. S. F. A. A-Z-III
- } Saccharin-Fabrik Aktien-gesellschaft vorm Fahlberg, List and Company, Magdeburg, Südost, Germany.
5. S. I.-220.—Actien-Gesellschaft für Anilin Fabrikation, Wolfen, Kreis Bitterfeld, Germany.
6. Bayer Dust.—Bayer Company, Inc., New York City.
7. Semesan
8. Semesan Jr.
9. Du Pont No. 12
10. Du Pont No. 12 Bel
11. Du Pont No. 13-U. A.
12. Du Pont No. 18
13. Du Pont No. 37
14. Du Pont No. 45
15. Karasch Compound A.
16. Karasch Compound B.
17. Karasch Compound C.
18. Karasch Compound D.
19. Karasch Compound E.
- } E. I. du Pont de Nemours and Company, Wilmington Del.
20. Mercury "C."—Roessler Hasslacher Company, Perth Amboy, N. J.
21. "Wa-Wa Dust."—Chicago Process Company, Chicago, Ill.

The seed used was Oderbrucker barley (Wisconsin Pedigree No. 6, C. I. No. 1146), grown in 1924 by the Agronomy Department of the Wisconsin Agricultural Experiment Station on the West Hill Experiment Farm, near Madison, Wis.

METHODS AND RESULTS

The seed was dusted by placing 350 grams of barley in a two-liter Erlenmeyer flask and adding an even tablespoonful of the dust to be used. After shaking the flask sufficiently to coat thoroughly every kernel with the dust, the seed was poured into a coarse sieve to remove the excess dust. The seed was then packeted and sown in triple row rows in two parallel series (Series I and II) and each series replicated three times. Series I was sown on the Arlington Experiment Farm, Rosslyn, Va., March 30, 1926. Series II was sown on the Wisconsin Agricultural Experiment Station Farm near Madison, Wis., two of the replications being sown on April 30, and the third on May 6, 1926. In both series, untreated controls were sown for purposes of

comparison. The rainfall and soil temperature data for the periods from a few days before the seed was sown until after the seedlings had emerged are shown in table 1.

Infection data were taken on Series I, June 9-12, 1926. In each of the triplicated rod rows, the plants in the middle row were pulled and the diseased and healthy plants counted. In the remaining rows only the striped plants were pulled and counted, the total number of plants in the middle rows serving as the basis for estimating the percentages of infection. A summary of the data is given in table 2.

TABLE 1.—*Rainfall and soil temperature data for the periods March 25 to April 15, 1926, at Arlington Experiment Farm, Rosslyn, Va., and April 24 to May 15, 1926, at Madison, Wis., in connection with experiments for the control of stripe disease of barley by the use of dust seed treatments.*

Series I, at Arlington Experiment Farm					Series II, at Madison, Wisconsin				
Date	Rain-fall in inches	Soil temperature in degrees C.			Date	Rain-fall in inches	Soil temperature in degrees C.		
		Maxi-mum	Mini-mum	Mean			Maxi-mum	Mini-mum	Mean
March 25		13	3	8	April 24	1.00	6	1	4
26	.29	8	1	4	25		12	0	5
27	.21	8	-1	3	26		7	2	4
28		8	-2	2	27	.09	11	2	6
29		11	-2	4	28		12	2	7
30*		12	-2	5	29		24	6	15
31	.43	7	4	5	30		28	8	19
April 1	.07	8	0	4	May 1 ^a		30	5	19
2		12	-1	5	2	.80	22	8	14
3		13	3	7	3	T	9	2	6
4	.02	12	1	6	4	.04	14	3	9
5		6	0	2	5		28	12	19
6		0	-3	-2	6 ^c		29	12	20
7	.16	10	-2	5	7		30	13	22
8	.17	10	5	8	8 ^b	T	25	13	19
9	.27	11	0	6	9		19	7	14
10		11	0	6	10		16	6	11
11		6	0	4	11		18	4	11
12 ⁺	.17	11	-2	5	12		20	6	13
13		8	3	5	13	.05	12	7	9
14	.02	15	4	9	14 ^d	.29	12	4	7
15		13	4	8	15		23	4	14

^a First series sown. ^b Emerged. ^c Second series sown. ^d Emerged.

T—Indicates trace of precipitation.

*—Date sown.

+—Date emerged.

DISCUSSION

The average percentage of infection in the controls in Series I was 19.47 per cent, while that in Series II was 12.77 per cent. The greater amount of infection in the former was due undoubtedly to the lower soil temperature prevailing during the period of germination, as shown in table 1.

Seven of the dusts used did not control the stripe disease sufficiently to merit further discussion. They were: S.F.A. A-Z-III, Du Pont No. 37 and Karasch Compounds "A," "B," "C," "D," and "E."

TABLE 2.—*Summary of data on the control of stripe disease in Wisconsin Pedigree No. 6 barley by the use of various proprietary chemical dusts. Seed sown in triple-
cated rod-rows replicated three times on Arlington Experiment Farm,
Rosslyn, Va., and at Madison, Wis., in the spring of 1926*

Number	Treatment	I. Arlington Exp. Farm			II. Madison, Wis.			Average per cent stripe
		Total plants	Striped plants		Total plants	Striped plants		
			Num- ber	Per cent		Num- ber	Per cent	
-	Control	2043	438	21.44	Average of 250 plants per row or 2,250 plants for each treatment and corre- sponding control.	271	12.04	16.74
1	Abavit "B"	2475	1	.04		5	.22	.13
2	S.F.A. No. 225	2421	29	1.20		49	2.18	1.69
3	S.F.A. No. 225V	2212	6	.27		13	.58	.43
4	S.I. 220	2178	8	.37		53	2.36	1.37
5	Bayer Dust	2583	28	1.08		41	1.82	1.45
6	Semesan	2493	24	.96		18	.80	.88
-	Control	2160	405	18.75		313	13.91	16.33
7	Du Pont No. 12	2538	5	.20		1	.04	.12
8	Du Pont No. 12-Bel	2475	30	1.21		15	.67	.94
9	Du Pont No. 13-U.	2601	12	.46		14	.62	.54
10	Du Pont No. 13-U.A.	2952	13	.44		22	.98	.71
11	Du Pont No. 18	2610	31	1.19		41	1.82	1.51
12	Du Pont No. 45	2196	3	.14		10	.44	.29
	Control	2016	368	18.25		278	12.36	15.31
13	Mercury "C"	2700	4	.15	14	.62	.39	
14	"Wa-Wa Dust"	2376	1	.04	0	.00	.02	

"Wa-Wa Dust" almost eliminated the stripe disease, only one infected plant being found in Series I and none in Series II. Abavit "B" and Du Pont No. 12 also were very effective, each allowing a total of only six striped plants to appear in the combined series. Six of the other dusts reduced the amount of infection in both series to less than one per cent. The plant counts in Series I (table 2) indicate that the treatments also improved the stand.

As shown in table 1, sufficient rain fell shortly after Series I was sown and after sowing the first two replications of Series II almost to saturate the

soil, as at the time of sowing both at Arlington Farm, Va., and at Madison, Wis., the soil already contained considerable moisture. This abundant soil moisture during the germination period may have increased the effectiveness of the dusts and consequently may have brought about better stripe control than would have been the case if the soil had been relatively dry. Controlled soil-moisture experiments are necessary to determine whether this is the case. In addition to this, it also will be desirable to test the effectiveness of these dusts on other varieties of stripe-infected barley before they can be recommended to take the place of the liquid treatments for the control of stripe disease. The possibility of soil reaction influencing the results also should be considered.

Therefore, while the results with certain of these chemical dusts for the control of stripe disease are very promising, additional experiments are needed to determine whether similar results would be obtained with different soil conditions and with other varieties of stripe-infected barley.

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THE OCCURRENCE OF BLAKESLEA TRISPORA THAXTER IN THE DUTCH EAST INDIES

S. C. J. JOCHEMS

Thaxter (4), in 1924, described a zygomycete of very interesting morphology which he had encountered as an impurity in a culture of *Botrytis rileyi* from Florida. He named the organism *Blakeslea trispora*.

This fungus, so far the only known species of *Blakeslea*, is very closely related to *Choanephora*. The latter is distinguished by conidiospores and sporangiospores. *Blakeslea*, on first view, is similar: its cultural characters are much like those of a *Choanephora*. On further investigation, however, it can be seen that the conidia of *Blakeslea* are in reality small sporangia, each with a small number of spores—usually four, although the number varies from three to seven. Thaxter called these sporangia sporangiola.

In addition to a detailed description, Thaxter included a few very clear drawings showing the morphology of *Blakeslea*.

Concerning the distribution of the mold, he only says:

“This interesting type, which has been named in honor of Professor A. F. Blakeslee, in recognition of his brilliant researches on the Mucorales, appeared as an impurity in a transfer of *Botrytis rileyi* which was kindly sent me several years ago, together with specimens of the affected larvae, by Professor Fawcett. The larvae attacked by the *Botrytis* were found on cowpeas at Gainesville, Florida, and it seems probable that the spores of the present fungus, which may have been growing on the faded flowers of this plant, were accidentally transferred to the diseased insect.”

Our phytopathological researches on Deli tobacco during the last three years have led us to believe that *Blakeslea trispora* is extremely common on the East Coast of Sumatra. Although our investigations so far have been confined to this region, we are convinced, as we shall show later, that it is distributed at least over all the Dutch East Indies.

We first encountered *Blakeslea* on faded tobacco leaves (Fig. 1) and particularly on those leaves which were infested by the great green tobacco bug (*Nezara viridis*). As a result of the infestation by *Nezara*, the leaf partly loses its turgescence and never recovers. Exactly how this takes place is not known, but we do know that some days after the leaf is infested, it becomes very weak. *Blakeslea* is then able to infect the leaf and hasten the



FIG. 1. *Blakeslea trispora* on tobacco leaf.

dying-off process. We have seen such heavy attacks of *Nezara* on tobacco estates that not only one top leaf wilted, as is usually the case, but that entire tops of many tobacco plants which were growing closely together began to fade. Parts of the wilted leaves were covered so thickly with *Blakeslea* fructifications that at one time we thought the mold was the primary cause of the wilting. However, we consider the *Blakeslea* as only a weak parasite, because we have never been able to obtain infections on healthy tobacco plants. The same is true of *Choanephora infundibulifera*, which is sometimes quoted as a parasite of the Hibiscus flower (2).

In addition to finding *Blakeslea* on faded leaves of the tobacco plant, we found it in drying sheds on newly plucked leaves whose tissues were still green and alive. The mold is also to be found in extraordinarily large quan-

tities, in Deli, on the plucked young flower-plumes which are broken off in the Delian tobacco culture and are to be found, sometimes in great quantities, on the ground under the plants. A few days later, particularly in the early morning, these are covered with the white bedewed fructifications.

In addition to tobacco, we found this mold on faded leaves of *Physalis angulata*, *Ipomoea batatas* and probably on some other plants. On these plants, mixed with the *Blakeslea*, there appeared another *Choanephora cucurbitarum*, which we described some time ago in an article on *Amaranthus blitum* (= *A. lividus* L.) (3). On this plant *C. cucurbitarum* is a true parasite. It causes the branches to die off, or, if the attack is very severe, kills the whole plant. On tobacco we have never been able to find it as a true parasite.

In our cultivation experiments (mostly on boiled, sterilized rice) and studies of the morphology of our fungus, we found a great deal of resemblance to *Blakeslea trispora*, described by Thaxter. The only point of difference was the number of spores per sporangium. Thaxter gave from three to six with a total of three in most cases, as he indicates in the specific name. We nearly always found more than three, in most cases four, although the number varied between three and seven.

Gandrup is the only one, who, according to Ultée (5), appears to have seen this fungus in the Dutch East Indies and made mention of it. An early annotation concerns a *Choanephora* variety in cultures originating from diseased tobacco bibit. The mold was noticed also in the plant-beds. The symptoms were the same as those which resulted from infection by *Phytophthora nicotianae*. Healthy bibit in pots was inoculated with this *Choanephora* species but in no case was infection obtained. According to Gandrup, the fungus was a new variety, and he named it *Choanephora dichotoma*. A further description will be given at a more convenient time.

In a second annotation, Gandrup (1) mentions among the molds obtained in pure culture from drying tobacco leaves, in addition to *Choanephora infundibulifer* (Cunn.) Sacc., a new *Choanephora dichotoma*. According to him, the *Choanephora* varieties grew only on freshly-plucked leaves which were not yet dead.

On the basis of further information from Mr. Gandrup himself concerning the morphologic qualities of the above *Choanephora dichotoma*, we are convinced that he was referring to *Blakeslea trispora*.

When one considers that *Blakeslea* appears in the entire tobacco district of East Sumatra, in a climate of equal warmth and rain throughout the year, and that it probably also can be found in a climate which is very dry during one-half of the year, as it is in the east corner of Java, it seems safe to conclude that *Blakeslea* is distributed throughout regions of intermediate

climate situated between these two. Research undoubtedly will establish the presence of this interesting fungus elsewhere in the tropics.

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APPLE BLOTCH CANKER ERADICATION¹

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By the omission of the blotch sprays from blocks of bearing trees in two young apple orchards of the very susceptible variety, Oldenburg (Duchess), at Vincennes, Indiana, the effectiveness of early canker eradication and prevention has been demonstrated. The methods employed and the earlier stages of the work in these orchards have been described previously.³ Briefly, the process consisted in shaving off or pruning out the old cankers of the blotch fungus (*Phyllosticta solitaria* E. and E.) and spraying to prevent the formation of new cankers.

One of the unsprayed blocks was located in a small planting of 156 Oldenburg and 61 Transparent trees set out in 1918, and the other in a large orchard of about a thousand Oldenburg trees set out in 1917.

In the small planting, 135 of the original 156 Oldenburg trees were left in 1925 and 1926. In 33 of these no cankers had ever been found, and from the other 102 trees about 114 cankered limbs had been pruned out and about 561 cankers shaved off since this work was started in 1922. In 1922, about 74 cankered limbs and 201 cankers were removed from a total of 66 trees. In 1923, 18 cankered limbs and about 154 cankers were removed from 62 trees, in 21 of which infection had not been previously detected. There were 19 cases of marginal renewal of fungous growth about old cuts, representing about 9 per cent of the old cuts. In 1924, 9 cankered limbs and 43 cankers were removed from 26 trees. In 1925 and 1926, 13 cankered limbs and 112 cankers were removed from a total of 61 trees, in 14 of which the disease had not been previously noted. However, 46 per cent of the 88 trees previously operated upon were free from infection. There were 32 cases of renewal of mycelial growth about old scars, usually marginal and at the side, occasionally within the scar. These represented about 8 per cent of the cuts previously made.

¹ Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Ind.

² The writer desires to acknowledge his indebtedness to Prof. H. S. Jackson for advice and to Mr. R. A. Simpson, Mr. Ivan Cushing and others in the Simpson Orchard Co., Vincennes, Ind., for cooperation and assistance.

³ Gardner, Max W. Origin and control of apple blotch cankers. Jour. Agr. Res. 25: 403-418. 1923.

The great majority of these cankers were the result of infection occurring before the campaign was started, and those found during the later years were invisible or overlooked during the earlier inspections. These results show the necessity of annual repetition of the process and emphasize the advisability of beginning the spraying and canker excision as soon as the orchard is set out rather than to give the disease three or four seasons in which to gain a foothold. The marginal renewal of mycelial growth about some of the cuts shows the importance of extending the cut well beyond the limits of the brown, discolored tissue, especially at the sides of the canker, since the mycelium tends to encircle the limb rapidly. It is important to remove all of the diseased tissue, but the cankers are shallow and can be shaved off without injury to the cambium.

Many of the cankers are difficult to detect, especially those on spurs, in crotches, and in the roughened, wrinkled bark about the bases of spurs. If a shallow cut is made with the knife, infection in such regions may be readily recognized by the brown discoloration of the outer bark tissues. Canker detection is best accomplished early in the spring before foliage and spray material interfere. The numerous short spurs on the interior of the tree are very likely to be infected and, being worthless, might as well be pruned out.

The proof of the effectiveness of this campaign was obtained when the orchard came into bearing in 1925. The special Bordeaux blotch sprays, which are put on usually two, four, and six weeks after petal-fall, had been applied annually since 1921, and in 1925 were omitted from a block of 68 trees comprising the eastern half of this orchard. After the petal-fall spray of lime-sulfur for scab control, only lime and lead arsenate were used. On July 10, shortly before the fruit was picked, all of the fruits on and under the 64 trees which bore fruit, 6,912 apples in all, were examined and no blotch lesions were found. Even though 1925 was a very mild blotch year because of the drouth in May, abundant infection occurred in older unsprayed trees in the vicinity; and in spray tests at Mitchell, 60 miles to the east, 85 per cent of the apples in an unsprayed check tree were infected.⁴ Therefore the non-occurrence of fruit infection in the unsprayed block was highly encouraging.

In 1926 another crop of fruit was produced and the blotch sprays were again omitted from the same block of 68 trees. On July 20 the fruit from 52 of these unsprayed trees was picked and left in crates under the trees, where it was graded for blotch infection. The fallen fruits and the immature fruits on the trees were also inspected. In all, 11,673 apples were

⁴ Gardner, Max W., Laurenz Greene, and Clarence E. Baker. Spraying tests for the control of apple blotch. Trans. Ind. Hort. Soc. 1925: 134-147. 1926.

inspected, and no blotch lesions were found. The 1926 season was much more favorable to blotch infection than that of 1925 according to the results obtained in spraying tests at Mitchell. Therefore the perfect freedom of the fruit from infection in the unprotected trees, 49 of which had once contained cankers, indicated that the sources of infection had been eliminated.

In the large orchard set out in 1917, the work of cutting out the cankers was begun in 1922 and was carried out by orchard men under the able direction of Mr. R. A. Simpson. The Bordeaux blotch sprays had been applied annually since 1920. A good crop was produced in 1925, and on a half row of 21 trees, 16 of which showed canker cuts, no blotch sprays were applied after the one applied two weeks after petal fall. Since tests carried out at Lafayette by E. J. Kohl showed that blotch infection occurred very late in 1925, the fruit on these trees was actually unprotected during the infection period. Just before the crop was harvested, 5,758 fruits on and under these 21 trees were examined and no blotch infection was found.

This orchard bore heavily again in 1926 and, after a lime-sulfur spray at petal fall, all of the Bordeaux blotch sprays were omitted from the same row of 21 trees. On July 21, 8,173 fruits on and under these trees were examined for blotch and only one lesion was found. These results indicate that canker eradication which had been started as late as five years after planting and which had been performed entirely by orchard men was remarkably successful.

The freedom of the fruit from infection in these unsprayed blocks for two years is considered to be very good evidence of the effectiveness of early canker prevention and eradication. How much might have been accomplished by the sprays alone had the cankers not been cut out is not known. However, the cankers are long-lived, and it was found in spray tests at Mitchell that three consecutive years of spraying did not eliminate the sources of infection in older, badly cankered trees.

Furthermore, the freedom of these unsprayed plots from infection is interpreted to indicate that, unlike the apple scab fungus, the blotch fungus is not usually disseminated long distances or from orchard to orchard by means of wind or other natural agencies and that its presence or absence in an orchard is largely determined by its presence or absence in the nursery stock originally set out.

Because Oldenburg nursery stock is so commonly infected and because the cankers increase so rapidly in this variety, its widespread use as a temporary filler between the permanent rows is believed to be responsible for the introduction of blotch into many orchards. Therefore it is important to start early to combat the blotch menace in this variety. A timely and thorough effort to eliminate the cankers will render the disease very easy to con-

trol by the time the trees come into bearing, and may possibly permit the ultimate omission of the special blotch sprays.

In summary it may be said that the effectiveness of blotch canker eradication in young apple orchards has been demonstrated by the freedom from infection of the fruit in blocks of trees left unprotected by the blotch sprays *for two years*.

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A MODIFIED METHOD OF DELINTING COTTON SEED WITH SULPHURIC ACID¹

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INTRODUCTION

The delinting of cotton seed with concentrated sulphuric acid has been strongly advocated for some time by workers at various Southern experiment stations interested in cotton culture. The method, apparently first used by J. F. Duggar² and outlined by F. M. Rolfs,³ consists in covering the seed with concentrated commercial sulphuric acid and stirring the seed continuously for 10 minutes. The excess acid is then drained off and used again, the process being repeated until the acid becomes too thick, which happens when it is used for the third or fourth time. After the seed is treated, it is thoroughly washed in running water for about 20 minutes, or until the disappearance of the acid taste. The seed is then spread out to dry.

The advantages of the delinting of cotton seed are as follows. First, certain fungi and bacteria parasitic on the cotton plant, such as those causing cotton anthraenose, wilt, angular leaf-spot, and some forms of damping-off, are not infrequently carried on the surface of the seed. When there is lint on the seed, the common chemical disinfectants are not quite effective. The treatment with sulphuric acid destroys not only the lint but also the spores of fungi and bacteria on its surface. Delinting therefore decreases some of the diseases and practically controls others, as is claimed in the case of angular leaf-spot. Second, the delinted seed is easier to handle in planting, and therefore its use would result in economy of both labor and seed. Third, the treated seed absorbs moisture more easily from the soil, and hence germinates more quickly, at least under conditions of sufficient moisture.

Brown and Gibson,⁴ discussing the present status of the treatment, say, "Until the present time, so far as the writers are aware, the method de-

¹ A brief, popular paper, based on the same experiments, was published as Circular No. 3 of the Tenn. Agr. Exp. Sta., 1926.

² Duggar, J. F., and E. F. Cauthen. Experiments with cotton. Ala. Agr. Exp. Sta. Bul. 153. 1911.

³ Rolfs, F. M. Angular leaf-spot of cotton. S. Carolina Agr. Exp. Sta. Bul. 184. 1915.

⁴ Brown, J. G., and Frederick Gibson. A machine for treating cotton seed with sulphuric acid. Ariz. Agr. Exp. Sta. Bul. 105: 381-391. 1925.

scribed by Duggar for delinting cotton seed is the only one that has been published. The most serious objections to this method are the slowness of the process, which involves considerable time and labor, and the danger accompanying the handling of concentrated sulphuric acid in open vessels. These two considerations have prevented the adoption of the sulphuric acid treatment to such an extent that it is seldom used except by experiment station workers." For these reasons they designed a machine which "delints the seed quickly and satisfactorily in large quantities at a comparatively low cost," the reference being to the cost of labor and the cost of the machine when large quantities of seed are treated. However, the principles of the treatment—covering the seed with strong acid, draining the acid to be used again, and afterward washing the treated seed—are left as in the original Duggar method. Therefore the cost of the treatment, so far as the acid is concerned, is not reduced by the use of the machine. Nor is the necessity for subsequent washing of the treated seed overcome.

While entirely agreeing with Brown and Gibson that the slowness of the treatment without the machine and the dangers involved in the handling of concentrated sulphuric acid in open vessels are serious objections, the writer considers that one of the chief objections also is the large amount of sulphuric acid needed for the treatment. This objection was of course not so serious with Brown and Gibson, when the cost of their acid was considered as \$16.00 per ton, or 0.8 cent per pound. Under ordinary conditions, and especially when the machine for the treatment is not available, the cost of the acid is by no means small. For instance, the writer's most recent information shows that acid in carboy lots is 6 cents per pound, or, on the basis of Brown and Gibson's figures, the cost of sulphuric acid for the treatment of a quantity of seed sufficient to plant an acre in Tennessee would be, not 14 or 15 cents, but nearly \$1.00, in addition to the cost of the machine and labor. To the writer it has always seemed that the amount of acid required by the original method was excessive and could probably be materially decreased by using just enough to delint the seed properly, avoiding the excess of acid which accompanied the method. In this way is eliminated also the draining off of the excess acid.

There are, of course, objections to the washing of the seed. It involves an additional handling of sulphuric acid, though diluted; it necessitates the subsequent drying of the seed, with all that this involves; and finally, in spite of instructions, it is probably seldom that the washing is sufficiently well done, and as a consequence the seed is injured. It is evident that instead of the acid being washed off it could be neutralized by an application of the proper substances. It was doubtful, however, whether such treatment would be practicable.

To determine the above questions the author, early in 1926, undertook certain experiments, the results of which are recorded here.

THE AMOUNT OF CONCENTRATED SULPHURIC ACID REQUIRED IN THE STANDARD METHOD

In the delinting of cotton seed by covering it with concentrated sulphuric acid, the writer found, first, that the same acid could be used four times, after which it was too thick for further use; second, that one part of sulphuric acid will treat six and one-half parts, by volume, of cotton seed.

THE MINIMUM AMOUNT OF CONCENTRATED SULPHURIC ACID NEEDED FOR DELINTING

Experiments showed that the minimum amount of concentrated sulphuric acid required for proper delinting is one part of sulphuric acid to seventeen parts, by volume, of seed. For the delinting, measure off seventeen parts of cotton seed and add one part, by volume, of concentrated sulphuric acid; then stir continuously for 5 or 10 minutes. The amount of sulphuric acid at first appears quite insufficient, but after a minute or two of thorough mixing the seed becomes well covered with acid, and delinting is completed after the acid is allowed to stay on the seed for from 15 to 20 minutes. Sixty minutes of contact with the acid failed to injure the seed.

DELINTING WITH DILUTED SULPHURIC ACID

By testing different dilutions of sulphuric acid, the writer found that one part of concentrated sulphuric acid diluted with five parts, by volume, of water, and one part of this diluted acid added to ten parts, by volume, of cotton seed, will effectively delint the seed if the mixing is thorough, so that the acid will be evenly distributed over the seed. But the seed must be spread in a thin layer, in a dry place, and left for about seven days. Seed thus treated with diluted sulphuric acid was kept in the laboratory for over a month, and for a few weeks in the plot barn, without any injurious effect on germination. Various other dilutions and amounts of diluted acid were also tested, but the method given was found to be entirely effective and at the same time most economical.

SUBSTITUTION OF WASHING OF THE DELINTED SEED WITH A NEUTRALIZING SUBSTANCE

Only the liming of cotton seed after the treatment with sulphuric acid was tested. It was found to give as good results as a very thorough washing—no matter whether the seed was delinted by the standard method (covering with sulphuric acid), or with the minimum of sulphuric acid (one

part to seventeen parts of seed), or by being treated with 1:10 diluted sulphuric acid, at the rate of one part to ten parts, by volume, of the seed. In these tests sufficient air-slacked lime was used to cover the seed thoroughly.

THE EFFECT OF THE TREATMENTS ON THE SEED

The cotton seed delinted by the three methods, and subsequently either washed or limed, was germinated in the greenhouse. There was no difference in the time of germination among the different treatments or the checks. There was apparently a difference in favor of the treatments as compared with the checks in regard to the amount of blighting of the seedlings. No noticeable difference was found, however, among the different treatments in this respect. Cotton seed, one and two years old, treated with the diluted sulphuric acid, part of it washed and part limed, was tested in 1926 on a fairly large scale in the field.⁵ This test showed neither injury nor benefit from the treatments. It should be pointed out, however, in this connection that the moisture condition of the soil at planting time was exceptionally good; that the seed used was of high germination and relatively free from diseases; and, finally, that the season was rather unfavorable for either angular leaf-spot or anthracnose. In other words, the checks showed a remarkably rapid germination of the seed, an excellent stand of the plants and very little anthracnose and angular leaf-spot on the plants during the season. The treated plots were at least as good. Only with a less favorable condition for seed germination, poorer seed, and a season more favorable for the development of diseases, could the test give a dependable indication as to whether the treatments are beneficial, and to what extent. The literature on the subject leaves no doubt of the benefit of delinting.

COMPARATIVE ECONOMY IN SULPHURIC ACID REQUIRED FOR THE DIFFERENT METHODS OF DELINTING

As previously stated, the standard, or Duggar's, method of delinting cotton seed with concentrated commercial sulphuric acid requires one part of acid to six and one-half parts of cotton seed. Delinting by the addition of the minimum amount of acid requires one part of acid to seventeen parts of seed, and the method of delinting with the diluted acid requires one part of the concentrated acid to sixty parts of seed—all of the proportions being by volume for both the acid and the seed. These proportions, when calculated on the basis of weight—the specific gravity of the concentrated acid being taken at 1.84 and the weight of one bushel of untreated seed at 32 pounds—are as follows: for the first method, 1 pound of the acid to 1.8

⁵ In cooperation with Professor S. H. Essary, Botanist of the Tennessee Agricultural Experiment Station.

pounds of seed; for the second method, 1 pound of the acid to 4.6 pounds of seed; and for the third method, 1 pound of the concentrated acid to 16.1 pounds of seed.

CONCLUSIONS

The experiments in the delinting of cotton seed show that the most economical treatment is that with diluted sulphuric acid, which effects a saving of 89 per cent of the acid as compared with the standard method. If for any reason treating with strong acid is preferred, the treatment could be given with the addition to the seed of only the necessary amount of concentrated acid, or one part to seventeen parts, by volume, of seed, which requires 60 per cent less of the acid than the standard method.

After treatment by either method the seed can safely be limed instead of washed.

SUMMARY

The experiments here reported show that the standard, or Duggar's, method of delinting cotton seed with concentrated commercial sulphuric acid requires ten times as much acid as is needed when the delinting is done with diluted acid by the method devised during the experiments; and that the standard method requires two and one-half times as much sulphuric acid as is necessary when the acid is added to the seed in the minimum efficient amount, which is one part to seventeen of the seed, by volume.

None of the three methods of delinting, when properly carried out, injures the germination of the seed.

Liming the seed after delinting can conveniently and safely replace washing in any of the methods.

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PHOTOGRAPHING LIVING CONIDIA MOUNTED ON AGAR¹

C. D. SHERBAKOFF

In my studies of *Fusaria* I find that one of the most difficult parts of the work, and the part that takes the most time, is making good illustrations of the conidia. Ordinarily the illustrations are in the form of camera lucida drawings. As a rule, drawings have to be made from living material examined under an oil-immersion lens. The drawings, even for a person of experience and ability, require much time. Moreover, they unavoidably reflect too much of the individual's interpretation, which may render very difficult a proper comparison between the drawings made by different persons. Finally, it must be acknowledged that in many cases the drawings are so poor as to be of almost no practical value for the purpose intended. There is therefore a long-felt need of some method to replace or supplement that of drawing, which would give a true picture of the conidia and also save some of the drudgery and time. The use of a photographic process for this purpose naturally suggested itself, and, as a matter of fact, was employed by various workers. Some of them, Morris and Nutting,² for example, clearly realized the importance of the use of photomicrographs.

As ordinarily made, however, good photographs of the conidia in a drop of water are very difficult to obtain. The difficulty lay primarily in the fact that the conidia would not remain still for a sufficient length of time to admit proper exposure. Besides this, the conidia in water always occupy different planes and are turned in different directions, so that at best comparatively few of them can be found in focus without crossing others or being too crowded. Even when there is a minimum of water under the cover glass, and when the edges of the cover glass are well sealed with paraffin or a similar substance, the conidia exhibit some movement, especially the Brownian in the case of small conidia. When using water one is never sure that the conidia will remain stationary for the time required for a proper exposure.

Therefore, in order to utilize fully the advantage of the photographic method, it was necessary in mounting conidia to use some substance that

¹ The paper, under a slightly different title, was presented at the Sixteenth Annual Meeting of the American Phytopathological Society, Washington, D. C., Dec. 30, 1924, to Jan. 1, 1925. See "Use of agar blocks for photographing living spores." *Phytopath.* 15: 1. 1925.

² MORRIS, H. E., and GRACE B. NUTTING. Identification of certain species of *Fusarium* isolated from potato tubers in Montana. *Jour. Agr. Res.* 24: 348-349. 1923.

would be free from the objections mentioned. In other words, instead of a drop of water there should be used a transparent, solid substance, with a minimum of free moisture to keep the conidia in their original turgid condition and exclude the air around them, and at the same time be sufficiently firm to keep the conidia in place. A clear 2-3 per cent agar, as ordinarily used in mycological cultural work, is such a substance.

. The agar for this purpose was first used in a manner similar to that followed in some laboratories for the examination of spore material; *i.e.*, small pieces were cut from a poured petri dish. The use of agar in such form gives satisfactory results, as far as holding the conidia in a stationary position is concerned. But, owing to the uneven thickness of the piece of agar, it is almost impossible to find a sufficiently large field with all parts in focus at the same time. For this purpose agar blocks of even thickness are required so that both surfaces will be parallel to that of the cover glass upon which the conidia are mounted. Such agar sheets or blocks are readily prepared when a series of slides are used as a mould. The slides at the ends are kept separate by the insertion of other slides, preferably about $\frac{1}{2}$ mm. in thickness, and the whole placed on edge over a clean glass plate. To make sure that the agar sheets will be of the same thickness throughout, the thin slides are broken in two and the halves of the same slide inserted between the ends of the separating slides. Before pouring the agar into the spaces between the slides it should be cooled down to a point near solidification. At first only a small amount is poured; when that becomes hardened, a little more is added, and so on until the filling of the mould is completed. After the agar is thoroughly hardened, the end halves of the broken slides are pulled out, and the remaining slides, with the agar, are stored in a moist chamber for protection against rapid drying and dust. In preparation for photographing the conidia, the top slide is carefully taken from the agar sheet, and the agar cut once lengthwise and four times across, so that each sheet gives ten blocks for the mounting of the conidia. Three per cent water agar, cleared with the white of an egg, and filtered, is about the best for this purpose.

An excellent mount is obtained when a small mass of conidia is first placed on a cover glass in a droplet of water, or a very diluted stain, such as safranin, and evenly smeared over it. The conidia on the cover glass are allowed to dry almost completely, until the surface has a dull appearance, and are then turned down over the agar block on the slide. The drying of water, or of the dilute stain, draws the conidia into an even, single layer in which they are often arranged side by side, as if by hand (Fig. 1). This arrangement usually is undisturbed when the cover glass is carefully placed over the agar block, and it allows one to select for the photograph a field in which a considerable number of conidia over a comparatively small

area are in a fine position to show clearly their true and detailed morphology.

The agar blocks keep the conidia in the same relative position on the surface of the cover glass for a long time, so that even after many hours the material will be found in the same field. The drying of the agar block at the edges, however, affects the focus; and this should be taken into consideration when an oil-immersion lens is used and when the exposure is longer than 15 seconds. If the exposure is not longer than 30 seconds, the defect can be corrected by focusing to a point slightly deeper than that of the clearest focus. If a longer exposure is needed, either a readjustment of the focus should be made about every 15 or 20 seconds or the agar should be protected against drying by having its edges sealed with beeswax or paraffin.

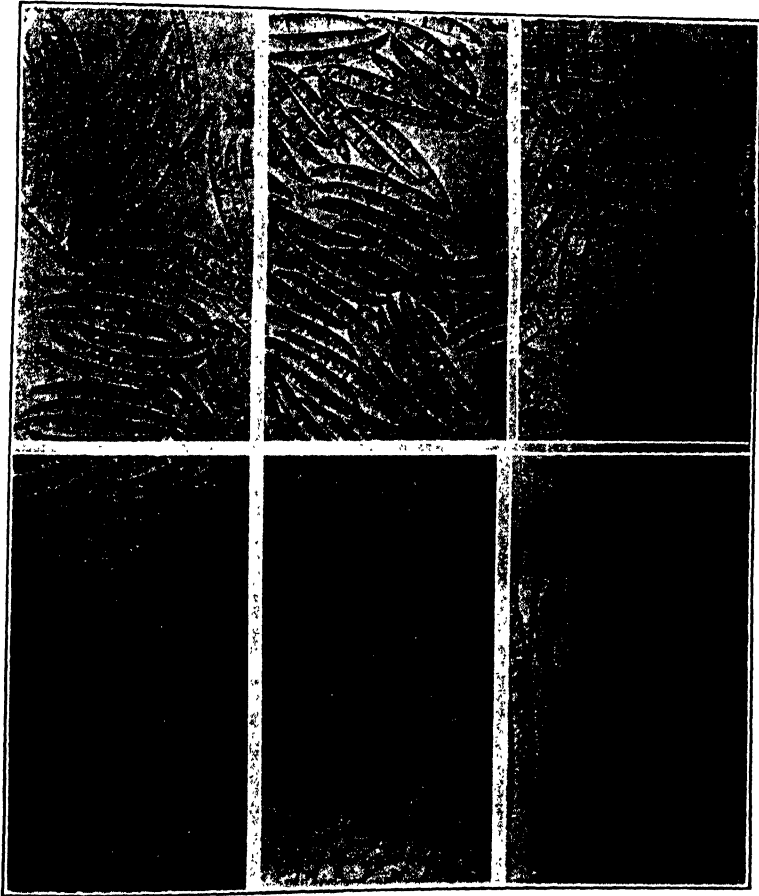


FIG. 1. A.—*Fusarium vasinfectum* Atk. B.—*F. oxysporum* Schl. C.—*F. culmorum* (W. G. Sm.) Sacc. D.—*F. solani* (Mart. pr. p.) App. u. Wr. E.—*F. martii* App. u. Wr. F.—*F. cumartii* Carp. Magnification 500 times.

I find, however, that a slight modification in the use of agar answers the same purpose and is less troublesome than the agar block with sealed edges. The modification consists in use of agar in a cavity of a slide. During the last two years it was found that the common, thin micro culture slides with spherical concavity are just as easy to handle as those with straight walled cavities, and are much better because of their relative thinness. Into the cavity of culture slide is placed melted, clear agar, just a little more than is necessary to fill the cavity, and a common slide placed over it. The process is then repeated until enough slides for several days' use are obtained. The slides should be warm, so that the agar will not solidify too quickly. A little excess of agar in the cavity is necessary to rid it of the air bubbles. The agar in the cavity is used in the same way as the ordinary agar blocks. The agar dries very slowly so that the focal position of the conidia, after the excess of water is absorbed by the agar, is not noticeably changed for at least two or three minutes. This permits the use of a slow plate, which gives the best results when sharp outlines of the conidia are required. If a still further limit to the drying of the agar is desired, the edges of the cover glass should be sealed with beeswax or paraffin. Dr. H. W. Wollenweber uses the following method: a small beeswax candle is lighted for a very short time, then the flame is put out, and the edges of the cover glass are smeared with the point of the hot wick.

The method of using agar mounts in photographing conidia or any other small objects is very simple, is available to all, and requires no extra equipment. At the same time it gives such satisfaction that in many instances photomicrographs will, I am sure, entirely replace, or at least largely supplement, drawings, with gratifying results. In my extensive use of agar instead of water for mounting spore material, I have developed the habit, so that now, even for camera lucida drawings, I use it exclusively. Taking certain sanitary precautions, and using a good moist chamber with a sufficient amount of filter paper moistened with a weak solution of corrosive sublimate, I often was able to keep the agar slides for several weeks.

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CONTROL OF INTERNAL ROT OF CAPRIFIED FIGS

H. H. HANSEN

About six years ago a new disease of the fruit of caprified figs was noticed in California.¹ Since then the disease has spread so rapidly that in 1926 it was found prevalent in all the fig-growing sections of the State, entailing considerable loss to the growers by reduction in quantity and quality of both fresh and dried fruit.

The etiology of the disease has been thoroughly worked out by Caldis, who named the disease "Endosepsis" (internal rot).² He found the causal organism to be a variety of *Fusarium moniliforme* Sheld. to which he gave the name *F. moniliforme* Sheld. var. *fici* Caldis. He further found that the pathogene occurs in the interior cavity of both capri and edible figs and that it is transmitted only by the caprifying insect *Blastophaga psenes* L. (*B. grossorum* Grav.). He proved by experimentation that transmission is entirely mechanical, the fungous spores being carried only on the exterior of the insect.

In view of the above facts it seemed that the only way to control the disease would be to obtain caprifigs and insects that were free from the fungus, propagate them in isolated localities and gradually caprify all other caprifigs in the State from them. A state-wide search was made and several thousand caprifigs were collected and tested, by caprification under controlled conditions and by culture in nutrient media, over a period of two years, but clean colonies were not successfully established.

When the writer took over the control problem in July, 1926, it occurred to him that it might be possible to apply to the interior of the caprifig a fungicide that would be strong enough to kill the fungus without injuring either the fig itself or the insects still in the galls. In order to test out this possibility the following experiment was carried out. A large number of caprifigs (Mammone) were treated internally with the following fungicides: mercuric chloride 0.05 per cent, formaldehyde 0.2 per cent, Mercurochrome 0.1 per cent, Semesan 0.2 per cent, and commercial lime-sulphur solution 5.0 per cent. Injections were made with a hypodermic syringe through the eye of the fig, not less than three weeks after the fig had been caprified, so that the insects would be either in the larval or pupal stage and therefore well

¹ Caldis, D. Panos. A rot of the Smyrna fig in California. *Science* 52: 161-162. 1925.

² Caldis, D. Panos. Etiology and transmission of endosepsis of the fruit of the fig. Hilgardia, Univ. of Calif. Press. [In press.]

down in the center of the gall. Treatment with the above mentioned fungicides had apparently no adverse effect on either the figs or the *Blastophaga*, as the figs matured normally and the insects emerged at the proper time.

Treated figs were removed from the trees and cultured on nutrient media at intervals of three weeks after treatment up to the time of maturity, when the emerging *Blastophaga* were caught and also cultured. An equal number of untreated figs, and *Blastophaga* from untreated figs, were cultured as checks. The following results were obtained: all the treated figs and the insects emerged from them were found to be free from *Fusarium moniliforme* Sheld. var. *fici* Cald.; all the untreated figs and the insects emerged from them were found to be 100 per cent infected with the fungus.

The above results seem to indicate that control may be effected economically by internal treatment of a limited number of caprifigs (preferably Mammone) with any of the fungicides which have been mentioned. By removal and destruction of all untreated figs, the source of infection would be eliminated and a clean strain of *Blastophaga* established.

This is a preliminary report on part of the work on fig diseases in general carried out under the direction of Professor Ralph E. Smith.

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FUNGICIDAL VALUE OF OIL SPRAYS

FRANK P. MCWHORTER

Since oil sprays have not been extensively experimented with by plant pathologists, the writer wishes to describe preliminary experiments which point to possibilities of their use in plant disease control.¹ In the vicinity of Norfolk, Virginia, where the humidity is relatively high, rose mildew caused by *Sphaerotheca pannosa* (Wal.) Lev. does great damage. It is almost impossible to grow successfully some of the more susceptible varieties of ramblers. Local conditions are such that commercial and home growers have found sulphur treatments very ineffective, in fact so unsatisfactory that this Station has ceased to recommend the sulphur treatment. This is explained in part by the frequency of rains at the time when mildew infections begin. Liquid Bordeaux is more effective when thoroughly applied, but most of the growers, especially the home growers, do not like to use it. With this situation in mind, the writer suggested the use of Volek concentrate mixture consisting of one pint of the concentrate to four gallons of water, the mixture to be applied coincident with the first appearance of mildew. This was tried by three growers who report remarkable control through its use. In each case three heavy applications were made at intervals of two to three weeks. The first application was made about six days after the mildew was first noticed. One large rambler which had not blossomed for three years, because of the disease, flowered abundantly in 1926. The grower believes that the treatment is responsible for the blossoming. One rose hedge about 110 feet long which has heretofore been unsightly and partly defoliated throughout the season has, apparently as a result of the treatment, kept its foliage and bloomed throughout the season. Untreated ramblers in the vicinity were heavily infected.

The exact nature of the fungicidal action has not been investigated. From general observations of the plants throughout the season the writer believes that the control evidenced is not entirely due to protection. One reason for this statement is that the majority of the patches of mildew covered with the oil failed to develop further. Obviously some smothering process is involved, but the possibility of a direct toxic action has not been studied.

¹ These experiments were undertaken upon information furnished by Mr. H. E. Woodworth, of the California Spray Chemical Company, who had noticed that certain fruit trees sprayed with emulsions prepared from "Volek" failed to develop mildew while those left as checks did.

Although the treatments were very heavy, no injury at all was observed for about four months after the first application. Leaf development was not checked, and most of the flower buds, even though heavily sprayed, opened normally. On September 20, some of the ramblers were showing some spray injury on the leaves, but not to an alarming degree. Since the plants usually keep their leaves but a short time after the first of October, the leaf injury factor will probably prove of little consequence. It is not comparable to a similar injury of citrus plants that sometimes follows too heavy applications of oil sprays, for in the case of citrus the leaves should stay on for a much longer time.

The results of the preliminary experiments mentioned above are so *promising that we are planning extensive experiments during the fall and spring with mildew and other diseases which have comparable infection processes. This note is presented with the hope that other investigators may find occasion to experiment with the fungicidal properties of oil sprays.*

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BOOK REVIEW

Citrus Diseases and Their Control. By Howard S. Fawcett, M.S., Ph.D., and H. Atherton Lee, M.S.: Large octavo, 581 pages. 205 plates and figures. Fifteen colored plates. McGraw-Hill Book Company, 370 Seventh Ave., New York City. Price \$5.00.

The senior author, Dr. Howard S. Fawcett, began his investigations on citrus diseases in Florida in 1905, and has given his uninterrupted attention to the subject since that time. In 1912 he transferred to California. In the meantime he has had the opportunity of making investigations in several foreign countries. The junior author, H. Atherton Lee, began plant pathological investigations in California in 1916, continued them in Hawaii, and later in the Philippine Islands. First hand field practise of the authors has enabled them to discard many useless and impracticable instructions and suggestions so frequently encountered.

This is the most lucid, accurate and comprehensive work that has been published on the subject. All known citrus diseases are included. The partially known or imperfectly investigated ones are noted and, as far as the literature permits, are discussed. The beginner in citrus pathology, in his efforts to extend the range of our knowledge, will find it an invaluable guide.

It differs materially from others that have been published on the subject in that the maladies are discussed from the viewpoint of the citrus tree rather than grouped artificially according to the classification of the causative agent. This manner of treatment makes the work especially helpful to the horticulturist and the practising plant pathologist. The authors deserve to be highly commended for having included only those remedies that practical experience has proved to be useful. Where trustworthy remedies are lacking, suggestions are made as to the directions in which experimenting may prove advantageous. The 78 pages of General Consideration contain information that is indispensable to every plant pathologist and to every citrus grower.

A novelty which will prove of great service to students in the colleges and to the practical horticulturist has been introduced in the form of a key for the determination of diseases, based on the region or part of the tree affected. The first division of this key serves to identify those maladies which affect the roots and trunk; the second, those which affect the branches; and the third, those which affect the fruits. The last section will prove an agreeable surprise to plant pathologists, as it illustrates and discusses fully the affectations to which fruit is subject after being removed from the tree, as well as those that may occur before clipping.

The value of the work has been greatly augmented by including a discussion of the affectations produced by animals other than insects, and by brief mention of some of the disorders caused by insects, which may readily be misinterpreted. A maze of difficulties which all students and even investigators encounter and stumble over is cleared up by the chapter entitled "Superficial Markings, Pustules, and Coatings." The pages on entomogenous fungi merit special mention. The authors strike out boldly and, justly, include such disorders as are caused by physical conditions and environment, as well as malnutrition, inherent genetic defects and physiological degeneration.

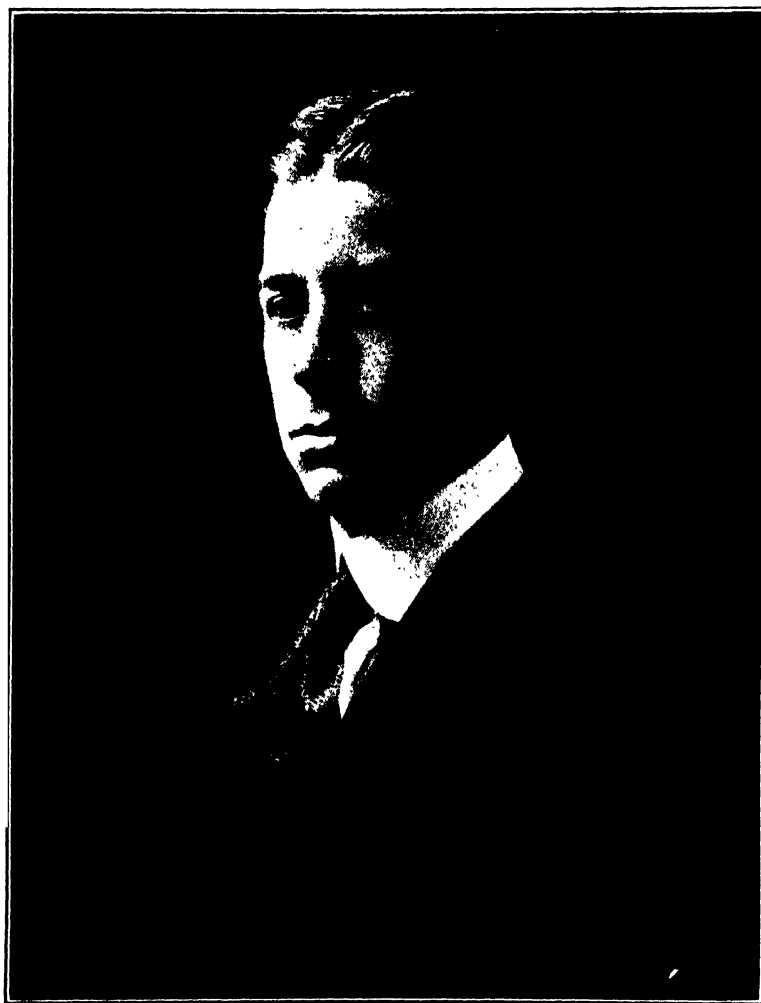
Over 130 maladies are discussed. The most important ones are illustrated, many by full-page, well executed half-tones, and about 25 by colored plates.

This unequalled treatise will give a vigorous impulse to the study of citrus diseases the world over, since it gives the investigator authentic information on all known diseases of citrus, and thus leaves him free to direct his efforts to imperfectly known and new diseases. The bibliography, which includes 467 citations, is a perfect mine of information for the investigator. The rapid progress being made in citrus pathology is indicated by the fact that 57 per cent of the literature cited has been published since 1915.

The mechanical work is about as perfect as bookmakers' art can produce. The heavy calendered paper used makes the half-tones stand out well. A few typographical errors occur, but none have been noted that are worthy of comment. The 15 color plates are well executed and add greatly to the value of the work, as they illustrate diseases and phases of diseases that could not be correctly illustrated by black and white prints.

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HAROLD WAKEFIELD FITCH

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HAROLD WAKEFIELD FITCH

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L. M. MASSEY

Harold Wakefield Fitch was born at Claremont, New Hampshire, June 5, 1897, and died at Grand Rapids, Michigan, May 16, 1926. After his graduation from the Claremont High School in 1916, he entered New Hampshire University, from which he received the degree of Bachelor of Science in 1921.

During the summer of 1920 Fitch served as Special Field Assistant in Albany County, New York, in which position he demonstrated marked ability in handling the applied phases of fruit disease control. In April, 1921, he was appointed to the Herman Frasch Fellowship in Plant Pathology at Cornell University and held this appointment until February 28, 1925, when he resigned to accept a position on the scientific staff of the Niagara Sprayer Company, with the privilege of returning to Cornell during the winters to work for his doctor's degree, for which he had practically completed the requirements at the time of his death. In this last position he had charge of demonstrational dusting work in several mid-western states, with headquarters at Grand Rapids, Michigan.

At Cornell, Fitch was engaged in testing the relative merits of dusts and sprays in the control of fruit diseases and he contributed much to our knowledge of this subject. His interest lay primarily in the applied field. He had a personality that inspired friendship and confidence, and a tireless energy that enabled him to conduct the extensive tests necessary in the arduous task of the orchard-testing of sprays and dusts. His characteristic devotion to his work, even to the neglect of his own health, was no doubt an important factor contributing to his untimely death.

His two papers, "Some results of dusting experiments for apple scab and peach leaf curl in 1921-22," published in *Proc. N. Y. State Hort. Soc.* 68: 42-60. 1923, and "Quantitative determinations of sulfur fungicides on foliage," published in *Phytopathology* 15: 351-354. 1925 (revised, *Phyto-*

pathology **16**: 427–428. 1926), are his most important contributions to the literature of plant pathology.

Never so rushed with work that courtesy was forgotten, never too busy to take the time that was necessary to offer sincere and sympathetic help to the grower with his practical problem, always pleasant and optimistic, with an enthusiasm for whatever he might undertake that completely overcame the monotony of routine tasks, Fitch endeared himself to all with whom he was associated.

A NEW SPECIES OF EXOBASIDIUM

J. W. HOTSON

INTRODUCTION

The examination of the genus *Exobasidium* reveals a great need for further cultural study of the species referred to it. The fact that the members of this genus are obligate parasites, or at least grow poorly on nutrient media, makes it very difficult to decide without host inoculation whether the various species listed are autonomous species or merely biologic forms. A careful and detailed account of the morphology and life history of *Exobasidium vaccinii* has been given by Woronin (10). This description has been based on observations extending over at least two seasons. Since his time, however, little attention has been paid to the morphology, although various new species have been described largely on the basis of form and color of the hypertrophy on new hosts or on the basis of a single or limited observation of an individual gall. It is quite probable, therefore, that there are some forms named which are not autonomous species. Of late years an effort has been made to rearrange these forms, grouping them mainly on morphological characters. Looking toward this end, E. Rostrup, in his Danish Fungi (4, pp. 350-352), divides the different types of *Exobasidium* in Europe into three morphologically distinct groups.

(1) The circumscribed type, which has its fruiting area on limited spots on the leaves, forming irregular gall-like bodies, each basidium giving rise to four small spores, $5-8 \times 1-2 \mu$. Apparently *E. vaccinii* (Fuck.) Wor. has been taken as the type for this group.

(2) The penetrating type, in which the mycelium penetrates either the whole host plant or single branches, causing hypertrophies on them and producing witches'-brooms, each basidium bearing only two large spores, $25-32 \times 8-12 \mu$.

(3) The *Arctostaphylos* type, intermediate between the other two, which occurs only on *Arctostaphylos*. This type is illustrated by *E. arctostaphyli* Harkn. with spores $12-17 \times 3-5 \mu$.

In the first group, Rostrup has apparently taken the spore measurements of *E. vaccinii* as given either in Saccardo's *Sylloge Fungorum* (5) or in Winter's *Die Pilze* (8), both of which, as has already been pointed out by Richards (3), are incorrectly reported. Instead of $5-8 \times 1-2 \mu$, the measurements should read $14-16.8 \times 2.8 \mu$ (10), or, as it is sometimes put, 14-

$17 \times 3 \mu$. On the basis of spore measurements, this correction would eliminate Rostrup's *Arctostaphylos* type and combine it with *E. vaccinii*.

Again in the second division the measurements of the spores should be, as explained by Burt (1, p. 655), $16-20 \times 7-8 \mu$, which corresponds to the size of the spores of the American collections of *E. vaccinii uliginosi*.

In America, Burt (1) has reduced the eleven species described as occurring on this continent to three autonomous species, the others being synonyms of one or another of these three. He sees no evidence that any of them are biologic forms. These three species are separated on purely morphological lines; the type of the hypertrophy and the host plays little or no part in the determinations. The first species, *E. vaccinii* (Fuck.) Wor., embraces all those forms in which the measurements of the basidiospores come within the limits recorded for this species, $14-17 \times 3 \mu$. This is the common and widespread species found in practically all parts of the United States where ericaceous plants grow. As synonyms of this species Burt has placed the following: *E. azaleae* Peck (*E. discoideum* Ell.), *E. rhododendri* Cramer, *E. peckii* Halst., *E. andromedae* Peck, *E. cassandrae* Peck, *E. arctostaphyli* Harkn., *E. cassiopes* Peck, *E. oxycocci* Rostrup, *E. karstenii* Sacc. and Trott (*E. andromedae* Karst, non Peck), *E. vaccinii myrtilli* (Fuck.) Juel. Of these the following have been reported from the state of Washington: *E. vaccinii* on *Vaccinium membranaceum*, *V. nevadensis* and *Rhododendron albiflorum*; *E. arctostaphyli* on *Arctostaphylos uva-ursi*; *E. cassiopes* on *Cassiope mertensiana*; *E. oxycocci* on *V. intermedium*; *E. vaccinii myrtilli* on *V. deliciosum*.

The second species, *E. vaccinii uliginosi* Boud., corresponds to Rostrup's second group and is distinguished by the fact that each basidium has only two spores, $16-20 \times 7-8 \mu$. This species has been collected on Mount Rainier by C. V. Piper (Acc. No. 443).

The third species is *E. symploci* E. and M., in which conidia only are known. It occurs on *Symplocus tinctoriae*, and has not been reported from Washington.

Burt has also found such a close resemblance of the morphological characters to those of *Corticium* and *Peniophora* that he has followed Saccardo's example and placed this genus in the Thelephoraceae rather than raising it to the rank of an order as Hennings has done in Engler and Prantl's "Die Natürlichen Pflanzenfamilien."

DISTRIBUTION AND HOSTS

The *Exobasidium* under consideration was first found in the spring of 1917 in the vicinity of Seattle, Washington, on *Vaccinium parvifolium* Smith. Since then it has been collected on the same host on Bainbridge

Island, Washington, by the writer; on Whidby Island, Wash., by Lena Hartge; near Snoqualmie Falls, King County, Wash., by Howard and Charles Gray; in the Elwha Valley, Olympic Mountains, Wash., by E. Hopf; and on Alsea Mountain, Oregon, by S. M. Zeller, and again by C. E. Owens. On April 25, 1926, it was collected by T. C. Frye near Snoqualmie Pass, Wash., on *V. ovalifolium* Smith.

The observations on this fungus up to 1921 were reported before the Pacific Division, American Phytopathological Society (2).

THE GALL AND APPENDAGES

During the first year of infection the stems are only slightly hypertrophied, but gradually increase in size from year to year, eventually forming a well pronounced gall (Fig. 2). The galls are firm and made up of hypertrophied host tissue with the perennial mycelium growing between the

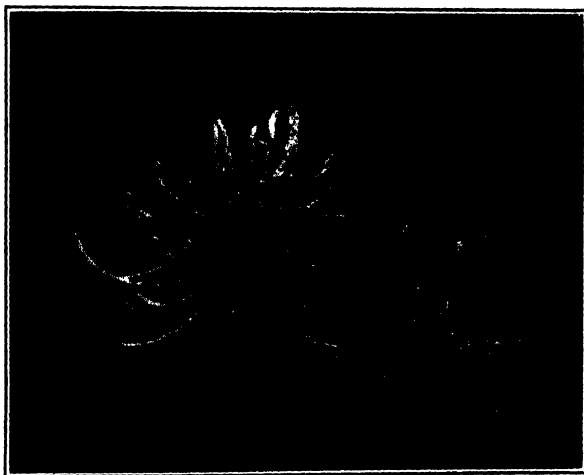


FIG. 1.—Mature appendages of a gall on *Vaccinium parvifolium* infected with *Exobasidium parvifolii*.

cells. They seldom completely girdle the stem so that the branch is not usually killed immediately. The longevity of the parasite is intimately related to that of the host branch on which it grows. The death of the host branch is immediately followed by the death of the fungus, there being no evidence that the mycelium lives as a saprophyte on the dead branch.

Each spring, in Seattle, about the middle of April, or a little earlier in some seasons, large numbers of soft, fleshy, more or less horn-shaped processes or appendages grow out from all parts of this gall (Fig. 1). These are usually single, occasionally branched, elongate, clavate, or more or less cylindrical in form (Plate IX, A). As they grow they pass through various

shades of pink, sometimes reaching a spectrum red (Ridgway). Later they become light green (Scheele's Green, Ridgway) without any evidence of the fungus on the outside. These appendages vary in length from 2 to 10 cm. and occasionally are as long as 15 cm.; the diameter is from 3 to 4 mm. or sometimes more toward the tip. The number of appendages depends upon the age of the gall. Over a hundred of these outgrowths have been counted on a single gall, while in another instance a single appendage was produced as the result of an infection at a leaf-scar. As these green processes approach maturity, which is early in June, they pass through various shades of color somewhat similar to those exhibited in their earlier stages of development. These changes begin at the tips and gradually work back to the base, the appendages eventually becoming completely white and often powdery (Fig. 1). Early in June, seldom later than the middle of the month, the appendages begin to turn dark and dry up, and in a couple of weeks all the beauty has disappeared and nothing but a few dark, thread-like strands are attached to the gall (Fig. 2, B). Thus from about the middle of June throughout the rest of the season the galls are so inconspicuous that they are easily overlooked.

STRUCTURE OF THE APPENDAGES

A microscopic examination of these fleshy processes shows that they are composed of the tissue of the host with the mycelium of the fungus growing between the cells. They are clearly outgrowths of the host, which has been stimulated to produce new cells by the action of the parasite. A comparison of one of these processes with a normal shoot shows a number of striking differences (Plate IX, B and C). In the first place, the shape is different. The normal young twigs of *V. parvifolium* are more or less four-sided with short spines over the surface, while the appendages are much smaller in diameter, more cylindrical in form, and without spines. There is also a marked difference in the color of the two. If cross sections are compared, it will be seen that the normal stem has one continuous ring of cambium, while in the appendage the vascular tissue is broken up into a number of more or less distinct parts (Plate IX, B and C). Moreover, in the appendages a partial ring of secondary bundles is produced in the cortex just below the epidermis, which, it may be noted, is not as regular or as definite as in the normal twig. By comparing B and C of the plate these points may be seen, as well as the differences in the general character of the cells that make up the ground tissue of each. It should be borne in mind, however, that the cross sections do not give the relative size. In reality the diameter of an appendage is much smaller than that of the normal shoot.



FIG. 2.—A. A gall with the appendages removed. B. A gall with only a few of the appendages removed.

THE MYCELIUM

The mycelium is perennial and intercellular in the cortex of the host. As the appendages grow out in response to the stimulation of the fungus, the mycelium keeps pace with them. The hyphae are very fine, about 1.5μ in diameter, and much branched. When these processes approach their maximum growth, the hyphae grow between the epidermal cells, here and there sending out branches that swell up, forming basidia which eventually produce basidiospores. The mycelium may live several years in the host. One gall has been observed to produce appendages for seven years. It would appear that at times the mycelium in the gall remains somewhat dormant, not producing sufficient stimulus to induce appendages to grow. In one instance, in the early spring of 1919, a certain gall failed to produce appendages and was considered dead. The next spring (1920), however, ap-

pendages began to grow out as usual, although in smaller numbers. In the spring of 1921 still fewer were produced, after which the gall apparently died as no appendages have been formed since. This, of course, may have been a case of reinfection, the mycelium of the original gall having died out during the winter of 1919 and the host reinfected during the spring or summer of the same year, but in that case the normal condition would have been to produce fewer appendages the first year and more the second—just the reverse of what happened. The evidence favors dormant mycelium as the better explanation. Moreover, as the infected branch becomes less and less vigorous the vitality of the parasite is correspondingly reduced. In this dying process it is quite possible that a point is reached where the mycelium does not produce sufficient stimulus to cause the appendages to grow but by the following year would regain a portion of its vigor.

BASIDIA AND BASIDIOSPORES

The basidia are formed by lateral branches of the hyphae pushing their way between the epidermal cells of the appendages and swelling. At first these project only slightly beyond the host cells, but at maturity often extend half their length. This portion, being relieved of the pressure of the epidermal cells, sometimes enlarges rather abruptly. The diameter ranges from 7 to 10 μ . The basidiospores are usually four on each basidium, sometimes five. They are elongated, non-septate, usually somewhat curved, finely granular, measuring $11-20 \times 2.5-4 \mu$ (Plate IX, E). Although the basidiospores are unicellular when mature, the first step toward germination is the formation of septa, usually three in number but not constantly so (Plate IX, F). No septate spores have been found attached to the basidium, but if left in water over night many of the spores become septate.

CONIDIA

Intermingled with the basidia but usually in large numbers are small acicular, one-celled conidia (Plate IX, G). These are formed singly at the ends of lateral branches but do not always inhibit the growth of the branch, so that occasionally they are found apparently lateral near the tip. The conidiophores often arise in tufts so that large numbers of conidia are formed in the same vicinity, giving a sort of mealy appearance to the horn-shaped processes. Whether or not these spores form part of the life cycle of the fungus under consideration could not absolutely be determined. They are, however, constantly, year after year, found associated with it on the same kind of mycelium, and under such intimate conditions that the writer has little hesitation in considering this spore-form as belonging to the life cycle of the *Exobasidium*. Inoculation experiments in which these conidia

were used failed to reproduce the disease. This, however, is not surprising, as the majority of experiments in which basidiospores and mycelium were used also failed. The mycelium producing the conidia can be traced back into the tissue of the host.

CULTURAL STUDY

Several attempts were made to grow the fungus on artificial media but with little or no practical results. Media made of potato, sugar, and stems of *V. parvifolium* were used, but no better results were obtained than Richards (3) got in his experiments with *E. andromedae*.

Better results, however, were obtained by making the cultures on living plants. In order that more galls might be obtained for future study inoculation experiments were made, first with *V. parvifolium*. Plants in a small ravine on Bainbridge Island were selected for this purpose. Deep in this ravine it is quite moist even in July and August. It was found that a very careful selection of host plants was necessary in order to insure success, as only those deep in the ravine and thus in a fairly damp situation became infected.

In the first experiments a few appendages containing mature spores were washed in sterile water, which was then put into an atomizer and sprayed over the whole plant. This method was not successful and was soon abandoned. The following method proved more successful. Portions of the appendages containing mature spores were applied to wounds in the host and then bound up, some with absorbent cotton, some without. These inoculations were made about the middle of June on (a) the current year's growth, (b) the last year's growth, (c) old stems, (d) leaves, and (e) roots. Of these, negative results were obtained on leaves and old stems; but infections were obtained on all the others, producing galls from which the characteristic horn-shaped processes grew out the next spring, few appearing the first spring, but the number increasing in successive seasons until the twig died or had lost so much of its vigor that the parasite died first, then the twig.

Having established the parasite on *V. parvifolium*, attempts were made to grow it on other species in the hope that it might produce some other kind of gall that might show its relationship to *E. vaccinii*. The available species for this purpose were *V. ovalifolium*, *V. ovatum* and *V. uliginosum*. Unfortunately *V. vitis-idaea* was not available for these experiments. Negative results were obtained in all attempts to inoculate *V. ovatum* and *V. uliginosum*. One typical gall was obtained on *V. ovalifolium*. As has been said, the fungus was later collected on this host near the Snoqualmie Pass, Washington.

DISCUSSION

As has been suggested, some species of *Exobasidium* have undoubtedly been described on too meager data, *i.e.*, on the characteristics of a single detached gall, or on observations for a single season without taking into consideration other forms of hypertrophy the parasite might produce. Obviously distinctive morphological characters are the most important factors in determining species, but they do not constitute the only characteristic to be considered. The relation of the species of *Exobasidium* to its host is comparable in some degree to that of a rust to its host. That is, the species of *Exobasidium* approach the condition of obligate parasites and not only the morphological characters but also the host and the effect on the host must be taken into account.

The size of the basidiospores of the *Exobasidium* on *V. parvifolium* come approximately within the limits given for *E. vaccinii*, but their shape is usually more cylindrical and rounded at both ends rather than spindle-shaped as reported for the latter species.

In searching the literature on this subject no description or illustration of a gall resembling this one has been found. The nearest approach to it is the "shoot gall" referred to by Burt (1, p. 629) which occurs on certain hosts of *E. vaccinii*. In this case, however, it is the lateral bud that grows out as a result of the stimulus of the parasite and produces a single gall, while in the species under consideration the gall does not necessarily arise from a bud but from any place where infection takes place, usually at some wound. Under the stimulation of the parasite the cambium is broken up and branches or shoots grow out, not one or two, but large numbers. The localization of the gall and the peculiar effect of the stimulus on the meristematic region of the host is unique. Moreover it is found on distinctly new hosts, and as far as is known it produces only one type of gall, there being no indication of hypertrophies of the leaves, buds, or flowers. For these reasons the writer is inclined to consider this fungus an autonomous species, and proposes the name *Exobasidium parvifolii* with the following description.

***Exobasidium parvifolii* n. sp.**

Mycelium perennial, much branched, intercellular, about $1.5\ \mu$ in diameter, producing cankers only on the stem or young branches from which grow out numerous fungus-permeated, corniform processes, 2–15 cm. long and 2–4 mm. in diameter; basidia hyaline, mostly claviform, 7–10 μ in diameter, formed on the surface of the corniform processes; sterigmata 4, sometimes 5; basidiospores cylindrical, hyaline, non-septate but becoming septate on germination, often curved, $11\text{--}20 \times 2.5\text{--}4\ \mu$; conidiophores unbranched,

similar to the hyphae, often in tufts; conidia simple, usually terminal, small, acicular, $5.5 \times 1.5 \mu$.

Hab. On the young stems (1–3 years old) of *Vaccinium parvifolium* Smith, in Washington and Oregon; on *V. ovalifolium* Smith, Snoqualmie Falls, Washington.

Mycelio perenni, ramoso, intercellularis, 1.5μ in diam. circa; carcinomatibus tantum in ramis junioribus ex quibus multi corniformes ramuli exerescunt intercurrentes inter cellos, 2–15 cm. longis et 2–4 mm. in diam.; basidiis hyalinis, plerumque claviformibus, $7-10 \mu$ in diam., formati in superficiei eorumdem ramulorum; sterigmatibus 4, interdum 5; basidiosporis cylindraccis, hyalinis, saepius curvatis, $11-20 \times 2.5-4 \mu$; conidiosporis simplicibus, verisimilibus hyphis, saepe cristatis; conidiis simplicibus plerumque terminalibus, parvulis, acicularis, $5.5 \times 1.5 \mu$.

Hab. In ramis junioribus *Vaccinii parvifolii* Smith, Washington et Oregon. U. S. Amer. bor. et *V. ovalifolii*, Washington, U. S. Amer. bor.

SUMMARY

1. A newly described *Exobasidium* occurs on the main stems or young branches of *Vaccinium parvifolium*, less frequently on *V. ovalifolium*, probably gaining access by some wound.

2. The mycelium is perennial, one gall having been observed for seven seasons, and several others from three to five.

3. According to careful observation for eight seasons, only the main stem, branches and roots are infected, with no indication of the disease on the leaves, buds, or flowers.

4. Large numbers of small, acicular, one-celled conidia are associated with this fungus.

5. The results of inoculation experiments thus far show that *V. parvifolium* and *V. ovalifolium* can be infected artificially and *V. ovatum* and *V. uliginosum* can not; that young branches up to three years old and roots are the only host parts infected; that the only kind of gall formed is that producing long horn-shaped processes; that these occur only in the spring from April to June inclusive; and that infection occurs most readily at wounds, even a slight wound such as a leaf scar being sufficient.

6. The name *Exobasidium parvifolii* sp. nov. is proposed and a formal description given.

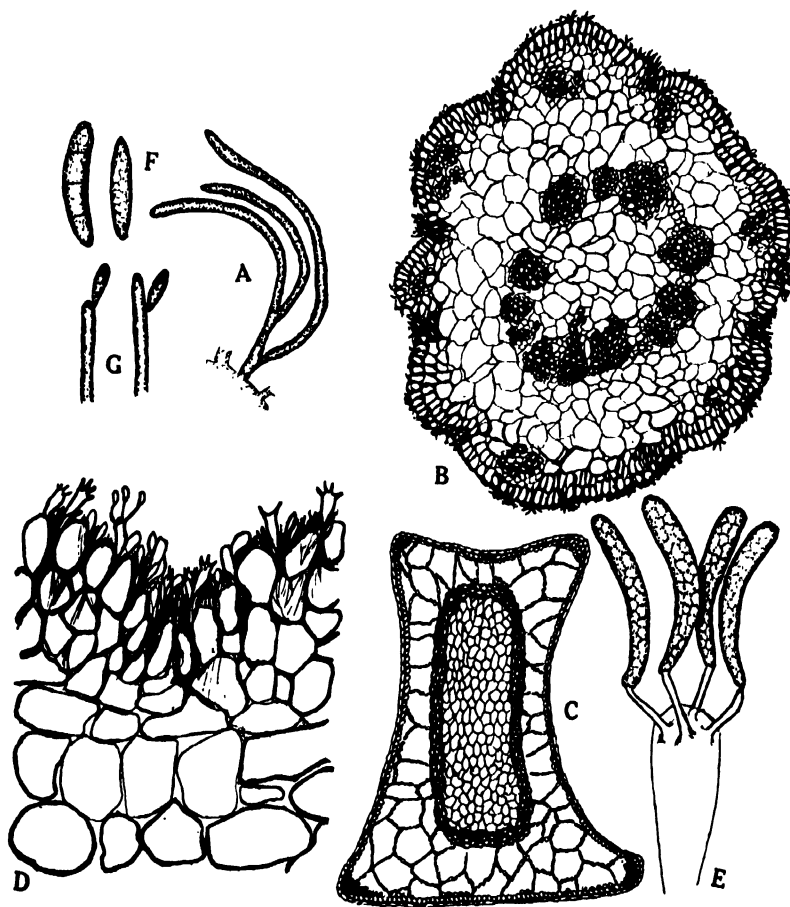
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EXPLANATION OF PLATE IX

- A. Horn-shaped appendages showing the infrequent branching.
- B. Cross section of one of the appendages showing the arrangement of the fibro-vascular bundles.
- C. Cross section of a normal branch of *Vaccinium* much reduced.
- D. A portion of one of the appendages showing mycelium, basidia and basidiospores.
- E. A basidium with basidiospores.
- F. Basidiospores.
- G. Conidiophores with conidia.



PALE DWARF DISEASE OF PEANUT (*ARACHIS HYPOGAEA*)

CARL HARTLEY

During the course of an investigation of bacterial wilt of peanut for the Instituut voor Plantenziekten, Buitenzorg, Java, in 1921-22, a type of dwarfing was encountered of which no description has been found in the literature. The name "pale dwarf" has been applied to it in order to distinguish it from club-leaf dwarfing, in which the chlorophyll content is above, rather than below, normal, and which will be described in another paper.

SYMPTOMS

In the pale-dwarf plants the early development of roots, hypocotyl, and stipules is normal or nearly so. The first leaflets are pale, much shortened, and their width more reduced than their length. The different segments of the same leaf are not always equally affected. In general, opposite leaflets are very much alike, but the distal pair is sometimes more reduced than the basal. In the most seriously affected plants the petioles are also greatly reduced. Subsequent development of roots and stipules is sub-normal, but only to the extent that would be expected as a result of the small carbohydrate production of the dwarfed top. Two extreme cases of the disease are shown in Plate X, A. The same abnormality, in a somewhat more typical form, is shown in Plate X, B. The habit of the plant is not greatly modified, the branches being no more ascending than are those of normal plants. The proliferation of leaves and stems reported recently in pale stunted plants in South Africa (1) has not been observed in the pale-dwarf plants.

In such cases as are shown in Plate X, A, the plant may live for a considerable time and put out successive pairs of tiny leaves, but is likely to die without recovery. Such a plant, at a later stage, is shown in Plate X, C, the photograph being taken 6 weeks after seed-sowing and just before the plant died. In cases not so severe, as shown in Plate X, D, and in the right-hand plant in Plate X, B, the leaves developed later are progressively more normal, until the plant is producing leaves entirely normal in size, shape, and color. Plants in which the leaflets of the first one or two pairs of leaves are one-half normal length and one-third normal width are soon scarcely distinguishable from normal plants. Such a plant as that in Plate X, D matures only a little later than the normal plants, but is small as a

result of the time lost at the beginning of the season, and produces few properly filled seeds. Between decidedly dwarfed plants and normal plants all gradations are found.

No curling or crinkling of the leaflets has been observed. With a single doubtful exception, the disease has never been found attacking a plant whose first leaves were normal. The typical course of the disease involves steady improvement. These characters distinguish the trouble very sharply from the African Kräuselkrankheit of Zimmerman (8, 9), which he says involves crinkling of the leaves, and from which a plant never recovers. While the Java krulziekte was described by Rutgers (4) as without crinkling of the leaves, it appears that cases of krulziekte often develop in plants which were normal in the juvenile stage; this, and the apparently deferred maturity and long continued flowering of the krulziek plants, argue against any relationship between Rutger's disease and the pale dwarf disease.

Dwarfing sometimes occurs as a result of infection with *Bacterium solanacearum*, but there is not the slightest possibility of confusing the bacterial dwarfing with the pale dwarf condition. The latter occurs both on wilt-sick fields and in plantings where the wilt bacteria do no damage.

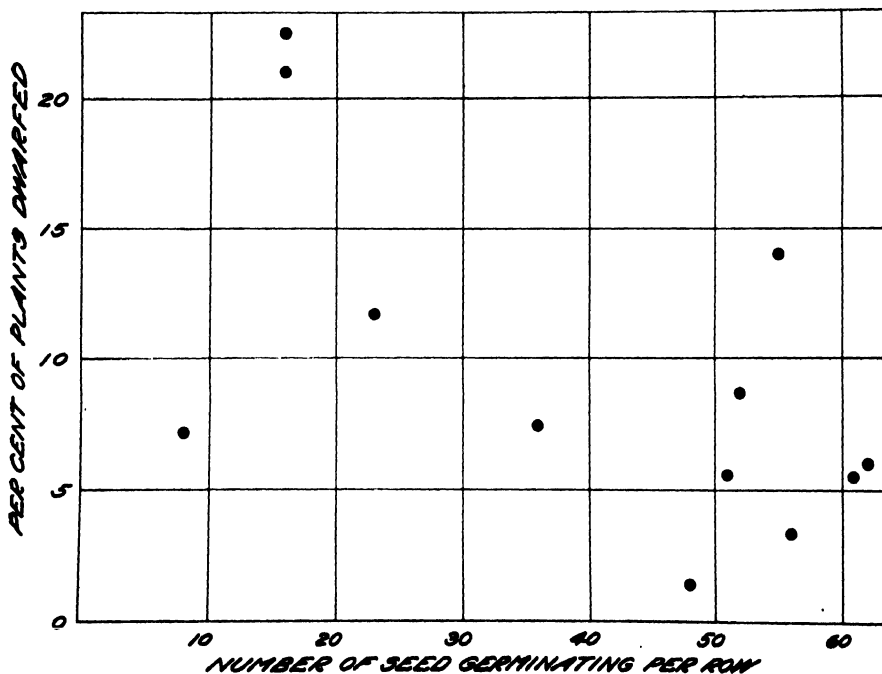


FIG. 1. The relation of germinative vigor to the incidence of pale dwarf in peanut seedlings of the Holle type, grown from seed received from East Java. Each point represents a seed lot from a different locality, grown in systematically replicated 2-rod rows.

IMPORTANCE

Pale dwarf plants were found everywhere in the West Java peanut districts, on both irrigated and unirrigated land and in both the East and the West Monsoons. In the plantings by the natives, the dwarfing was nowhere observed in sufficient amount to constitute a source of real loss. Of numerous plantings made in the writer's experimental work, in only four was it present to a sufficient extent to affect yield. Even in the experimental planting in which it was most prevalent, only one of the 32 seed lots with normal germination vigor produced as many as 10 per cent of plants with undoubted dwarf symptoms. The percentages of plants affected in each of the seed lots in this test are shown in figures 1 and 2. The dwarfed plants are scattered through the plantings. The loss or suppression of such scattered plants in the juvenile stage is approximately equivalent to an early thinning of the stand, as plants so much affected as not to produce a reasonable crop are also too small to have a material competitive influence on the rest of the stand. The space and materials which the dwarfed plants should have utilized are taken by the spreading out of neighboring plants, the spacing in Java peanut fields being much closer than is customary with

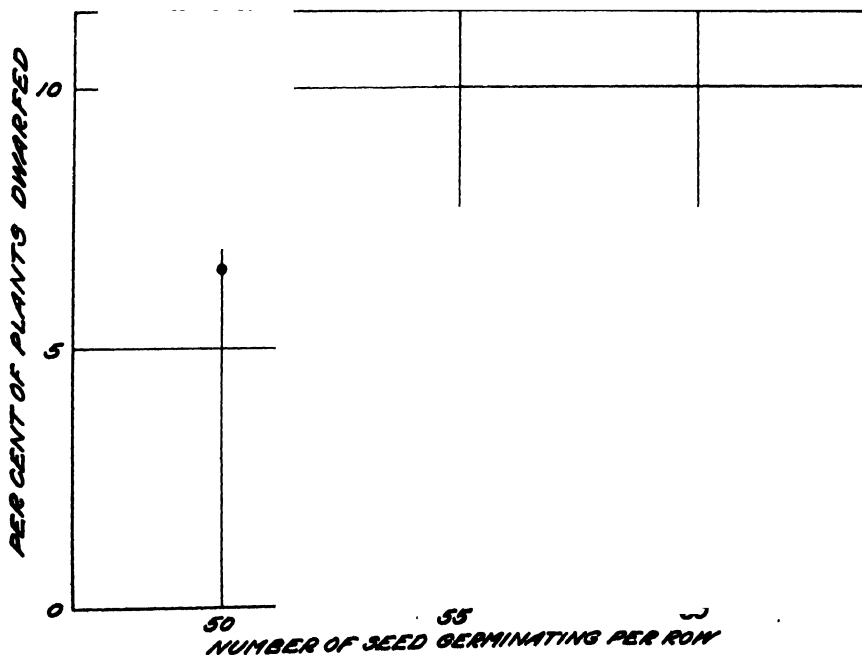


FIG. 2. The relation of germinative vigor to the incidence of pale dwarf in peanut seedlings of the Holle type, grown from seed received from parts of the Netherlands East Indies other than East Java. Each point represents a seed lot from a different locality, grown in systematically replicated 2-rod rows.

varieties of similar growth habit in the United States. Only in cases in which the stand is already unusually thin as a result of seed rot or drouth would the serious dwarfing of 10 per cent of the plants result in a reduction in the ultimate yield approaching 10 per cent.

There is another possibility to be considered in judging the importance of the disease; the association of poor germination with a high percentage of visible dwarfs may mean that the most seriously affected seedlings are kept from appearing above ground, or that the factor which causes pale dwarf can also prevent germination.

DIFFERENCES BETWEEN DIFFERENT SEED LOTS IN LIABILITY TO DWARFING

Tests were made with numerous seed lots of the two standard types of peanut, Holle and Broel, erect-growing types, which mature in 100 days in Java; the Broel has small pods and otherwise closely resembles the so-called Spanish variety grown in the southern United States. The incidence of pale dwarf in the two types was about equal. The same dwarf condition occurred in plants from seed coming from Menado, southern and central Sumatra, and all parts of Java. In addition, there were tested two lots each of the late-maturing prostrate type Tjina, and of a West African peanut intermediate in habit between the erect and prostrate Java types. Both of these types also produced distinct cases of pale dwarf, pictured for the African type in Plate X, B. So far as could be told from the small representation, the two latter types were quite as inclined to dwarfing as the Holle and Broel.

Within the Broel type, in the writer's experiments, the difference in dwarfing tendency between different seed lots was not great. This is in accord with the high degree of constancy found for the different Broel seed lots in the other characters for which they were compared. Within the generally more variable Holle type, it was very evident that different seed lots differed also in dwarfing tendency.

The differences between Holle seed lots in the percentages of dwarfs which they produced were apparently related to the germinative vigor of the seed, and to hereditary differences between different lots. The relation between dwarfing and germination will be considered in the next section. In the present section, comparisons will be limited to seed lots of approximately equal germinative vigor.

It was evident from the results of the experiment in which dwarfing was most prevalent that seed lots received from the Besoeki and Ngandjoek districts, East Java, were decidedly more inclined to produce dwarfs than were the seed lots from other districts. This can be seen by comparing the height of points of equal abscissal positions, figures 1 and 2. A number of these

East Java seed lots had deep red seed skins, and 3 to 4 seeds per pod. It seems more reasonable to attribute the high dwarf tendency of these seed lots to hereditary predisposition than to differences in the conditions under which the seed was grown, in view of the fundamental similarity in climate between the Besoeki and Ngandjock districts and the Mid-Java districts, from which a large number of the other lots in the experiment had been obtained.

Another indication of hereditary difference in liability to pale dwarf was found within one of the Besoeki seed lots. It contained both red and brown seed. When sown separately in parallel rows, the brown seed, although germinating better than the red, produced plants, 14.0 per cent of which showed pale dwarf effects, while the red seed produced only 1.4 per cent dwarfed plants. In every one of the 6 replications the brown seed row showed more dwarfing than the median of all seed lots for that replication, while the red seed row showed no dwarfing in 4 replications and, in the 2 remaining replications, less dwarfing than the median of all seed lots. This case is evidence that liability to dwarfing is not necessarily associated with red seed skin.

The relative freedom of the Toeban variety was, perhaps, the best evidence of hereditary differences in liability to dwarfing. Of the 25 rows scattered as checks through the experiment represented in figures 1 and 2, 20 contained no dwarfed plants and the remaining 5 practically none. The relative freedom of this variety was further confirmed in 18 rows of Toeban from another seed lot, scattered through another experiment in which were tested 16 additional seed lots not used in the first-mentioned experiment. In this test the Toeban remained entirely free from dwarfing, while 14 of the other 16 varieties were dwarfed. In a still later test, the results of which are shown in figure 3, three different lots of Toeban all showed less dwarfing than did most other lots of equal germination vigor. The percentages of germination of the three lots were 68, 81, and 91, and the percentages of plants dwarfed for the same lots 1.2, 0.6, and 0.3 respectively.

It seems reasonable to conclude that hereditary differences in liability to the pale-dwarf condition are not great, either as between different types, or between different seed lots within the Broel and Holle types. Within the Holle type there appear to be some differences, but much less marked than the hereditary differences, which are found within this type, in morphology and in resistance to *Bacterium solanacerarum*.

No evidence was obtained of transmission through the seed. Seed was obtained from 10 plants that had shown definite dwarfing in early life, and 15 others that had been marked as slightly or doubtfully dwarfed. This seed was sown in cool moist weather. In the 68 plants resulting, not a single case of dwarfing occurred.

RELATION OF SEED VIGOR TO DWARFING

The relation between germination and the incidence of pale dwarf in the seedlings was clearly seen for the Holle type in the results of two experiments shown graphically in figures 1-3. The Broel lots tested in experiments in which pale dwarf was prevalent were so nearly on a par in the matter of germination that no clear relation is evident. The position of the dot farthest to the left in figure 3 supports the expectation that in Broel, as well as in Holle, old seed of low germinative vigor will show the most tendency to dwarfing. In all three graphs the relation appears to be curvilinear.

RELATION OF PHYSICAL FACTORS AND DWARFING

Since the heredity and lack of vigor of the seed sown seem to be only loosely associated with dwarfing, it is necessary to look elsewhere for the real cause of the disease. As no good reason was found for supposing the trouble to be parasitic, the physical environment during the germination period was considered. Abundant dwarfing was found only in plantings which germinated during dry weather; the series showing the largest amount of dwarfing was, furthermore, one in which the seed had been covered less deeply than is usually done. This last finding is in disagreement with the belief of some of the agriculturists in Java, that the dwarfing

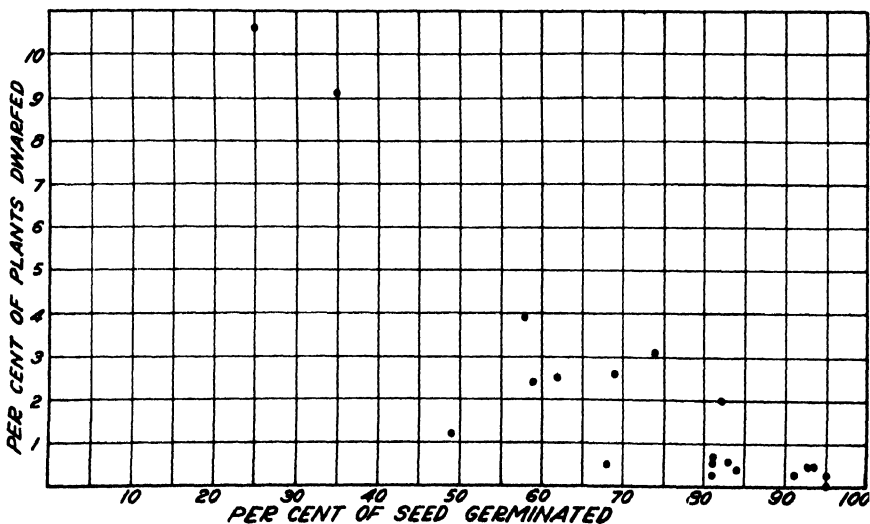


FIG. 3. The relation of germinative vigor to the incidence of pale dwarf in a later experiment. Each point represents a seed lot which was grown in 20 systematically distributed rod rows. The point farthest to the left represents a Broel type seed lot; the others were all of the Holle type.

is due to too deep planting. Both drouth and shallow planting are likely to result in unusually high temperature of the soil and seed during the germinating period. It is suggested that the direct cause of the dwarfing may be prolonged exposure of the sprouting seed to abnormally high temperatures, and the consequent breaking down or inactivation of some substance essential for the growth of the leaflets.

Further indication of heat relation was found in an experiment in which 10 plots were given especially deep cultivation, which resulted in very loose soil at the time of sowing. Ten other plots alternating with them were given little cultivation, and the surface soil was much more compact. As would be expected, the surface soil temperatures were higher in the loose-soil plots because of their poor conducting capacity. Counts showed 118 decided cases of pale dwarf in the loose-soil plots, and only 73 in the compact-soil plots.

Brief heating of the still-dormant seed does not, of necessity, result in dwarfing. In one of the experiments the seed was kept in glass jars during the sowing operations, and exposed to the direct rays of the sun. The seed skins on the side next to the glass soon became discolored, apparently because of heat, and the seed became very hot to the touch, but the amount of pale dwarf in plots sown with this heated seed was actually less than in the plots sown before the seed in the jars had become overheated.

In one experiment, dwarfing appeared in plants which were shaded by trees during a considerable portion of the day. This does not necessarily overthrow the overheating hypothesis. In the United States, pine seedlings have been killed outright by excessive heat, both in nursery beds (3) and forest plots (7), in soil which was exposed to the sun during only part of the day; and in German East Africa, partly shaded plants of *Cedrela odorata* developed typical heat lesions (5).

A somewhat similar case has been described in another plant species. In potato the spindling sprout disease was at one time thought to be due to overheating (6); it may also occur as a symptom of one of the infectious degeneration diseases. In the cereals, Crocker (2) quotes Haberlandt, without citation, as showing that the optimum germination temperature for wheat is 25° C., so far as germination itself is concerned; but credits Gutzeit with showing that, with many small grains, germination at temperatures above 20° C. gives weak plants and small yields, and that the optimum germination temperature from the standpoint of the ultimate yield is 17° C. It seems reasonable to believe that the attention which has been recently given to the killing of plant tissues by excessively high temperatures may well be extended to the question of harmful effects in cases in which there is no obvious necrosis.

Although the type of dwarfing here described has not been reported outside of Java, its presence in seedlings from African-grown seed gives reason for supposing that it will be found in countries other than Java if a search is made for it under proper environmental conditions. The possibility is not excluded that the disease is a manifestation of some systematic parasite which is able to produce recognizable symptoms only in young plants and under special environmental conditions. The probability is, however, that the trouble is entirely non-parasitic.

SUMMARY

1. A new juvenile disease of *Arachis hypogaea* is described under the name "pale dwarf." It is characterized by paling and marked reduction of the leaflets and, in extreme cases, reduction of the petioles. Roots, hypocotyl, and stipules are not dwarfed. Plants usually recover in later life, and mature at the normal time, but as a result of the early setback, their yield is reduced.

2. The disease is generally distributed in West Java, but is usually infrequent and of little economic importance. It has also appeared in seedlings grown in Java from seed brought from West Africa.

3. The incidence of dwarfing is consistently higher in the progeny of some seed lots than in others. This is partly due to the inverse correlation between germinative vigor and dwarfing tendency, but in part probably due to hereditary differences.

4. The dwarfing is believed to be non-parasitic in origin. There is some reason to suspect that it is due to excessive heat of the surface soil after seed sowing; it is suggested that this may destroy or inactivate some substance needed for the normal growth of the leaflets.

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EXPLANATION OF PLATE X

A. Two extreme cases of pale dwarf, from Java Holle seed.

B. Typical pale-dwarf plants, grown from seed imported from west Africa. The near-normal leaves on the right hand plant are on side branches, and were formed later than the much reduced leaves on the central stalk. The black strip shown for comparison is 1 cm. wide.

C. The extremely dwarfed plant in front of the card was less than 2 cm. high, although the photograph was taken six weeks after planting, and numerous pairs of leaves had developed. Although slightly larger leaves were beginning to appear, the plant decayed a few days later, presumably because of lowered resistance due to starvation. Leaves of a normal plant appear at the upper corner of the picture. The stake is marked in centimeters.

D. Holle plant which is producing near-normal leaves after being moderately but distinctly dwarfed in its early stages.



A DISEASE OF COTTON ROOTS PRODUCED BY FUSARIUM MONILIFORME SIELD.¹

NAOMI CHAPMAN WOODROOF²

INTRODUCTION

During the summer of 1920, and again in 1921, the agronomist at Georgia Experiment Station called attention to the very large percentage of dwarfed cotton plants in the fields. Such plants when pulled showed discoloration and in most cases a distinct girdling just below the surface of the soil. Specimens were received from Mississippi during the summer of 1921 with the statement that a very large percentage of the plants were thus affected and were setting few or no bolls.

The trouble was at first attributed to *Rhizoctonia*, but the appearance of the diseased area and the persistence of the dwarfed condition caused some doubts as to the correctness of the diagnosis, since plants attacked by *Rhizoctonia* are either killed outright or else recover and overgrow the lesions during the early stages of growth. It seemed likely, therefore, that some other organism might enter the *Rhizoctonia* lesions and produce the symptoms noted.

A number of dwarfed plants were brought to the laboratory during July, 1921, and the diseased areas examined microscopically for the presence of *Rhizoctonia*, but this fungus was not found in any case. Seventeen of the plants were placed in a moist chamber for 48 hours. At the end of this time examination showed a growth of *Verticillium*-like conidia and conidiophores over the surface of the lesions. A culture of this fungus was obtained and in preliminary inoculation tests was found capable of infecting healthy roots of seedling cotton plants.

The following year a more extensive study was planned. A number of fungi were isolated from the surface of the diseased plant roots. Further isolations were made during the summers of 1924 and 1925. Nearly 100 isolations were made from seedlings and older cotton plants.

INOCULATION TESTS

Pot Cultures. All preliminary inoculation tests were made in 6-inch pots of sterile soil which had been autoclaved at 20 pounds pressure for from two to three hours. Four pots were used for each test; in each pot 15 seed were planted. Four pots planted with sterile uninoculated seed

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• ² The work was carried out under the direction of Dr. B. B. Higgins, who made all notes and observations from 1920 until the summer of 1924.

were included as checks in each series of twelve pots. Thus there was a series of checks for each two groups of inoculated pots.

The seed were prepared for inoculation by delinting with sulphuric acid, removing as much as possible of the acid by washing in tap water, neutralizing the remainder with sodium carbonate and soaking in a 1-1000 solution of bichloride of mercury for 30 minutes followed by two washings in sterile water. The seed were inoculated by shaking in a spore suspension of the desired culture.

Fifty inoculation tests were made using thirty-three different cultures. Twenty-five additional tests were made but discarded in this summary either because they were examined before definite symptoms of the disease had had time to develop or because the check pots became contaminated.

For examination, the soil was washed from the roots of the plants, preserving intact as many of the lateral roots as possible. Counts were recorded and microscopical examinations made. Reisolations were usually made.

It was found that distinct dark brown lesions accompanied by typical girdling do not develop until the third or fourth week following planting.

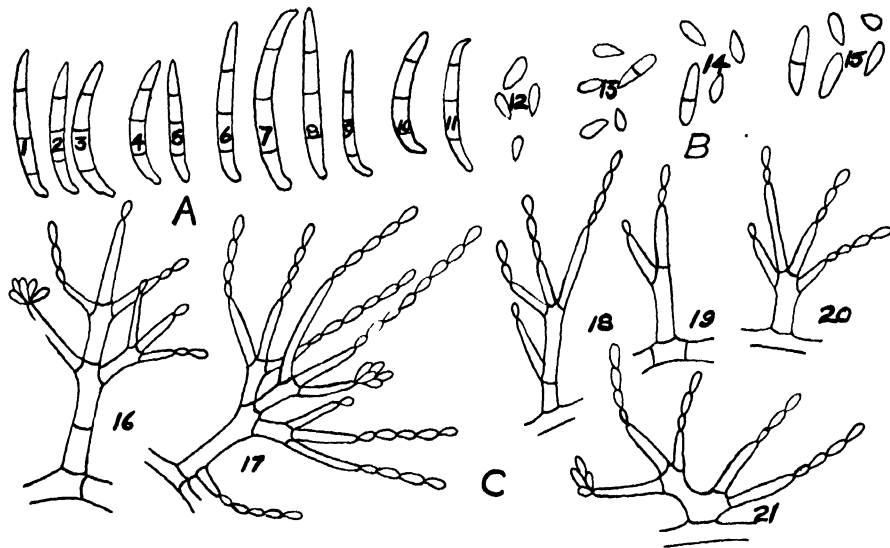


FIG. 1. Conidia and conidiophores of *Fusarium moniliforme* Sheld. Drawn with aid of camera lucida with a Zeiss 4 ocular and a B. and L. 4 mm. objective. A. Macroconidia: 1, 2, 3, from rice after 26 days; 4, 5, from Irish potato plugs after 18 days; 6, from bean pods after 5 days; 7, 8, 9, from Irish potato plugs after 9 days; 10, 11, from bean pods after 16 days. B. Microconidia: 12, from Irish potato plugs after 9 days; 13, from oat agar after 7 days; 14, 15, from bean pods after 12 and 9 days respectively. C. Conidiophores: 16, from cotton stems after 27 days; 17, 20, 21, from water agar; 18, 19, from potato agar after 20 and 7 days respectively.

Yellow discoloration and light brown initial lesions appear by the end of the second week. Figure 2 illustrates the difference in size and appearance of the healthy and diseased plants from the pot tests.

Eight of the thirty-three cultures tested were actively parasitic, all being capable of producing from 90 to 100 per cent infection. The remaining twenty-five cultures were either non-parasitic or only moderately so. All pathogenic cultures produced *Verticillium*-like microconidia and conidio-phores.

Bottle Cultures. Air-borne spores became a serious source of contamination after diseased plants had been grown in the greenhouse in pots for a period of time, even though the benches were thoroughly washed each time with a 1-1000 solution of HgCl_2 . It became impossible to produce clean checks. In order to overcome this difficulty, bottles of about 4-liters capacity were used. These were filled with moist soil to a depth of about 6 inches, plugged with cotton and autoclaved at 20 pounds pressure for one hour on two successive days. Seed were inoculated in the same manner as for the pots. Fifteen seed were planted per bottle. Four bottles were used for each culture and four uninoculated checks for each group of twelve bottles. Watering was not necessary, as the soil dried very slowly.

The disease developed somewhat more rapidly in the bottles than in the pots, but the symptoms in both were similar. The progress of the disease was observed on roots of plants growing along the sides of the bottles. The yellowing of the primary root and the destruction of the root hairs was followed by a period of a few days to two weeks during which time no visible growth was made by any part of the seedling. Finally, clean, white, lateral roots were pushed out from the discolored surface of the primary root and growth was resumed, but the plants remained smaller than those in the check bottles. In like manner the lateral roots became infected after the development of the root hairs. Thus the usual rapid extension of the root system was prevented.

✧ *Field Inoculations.* In the spring of both 1925 and 1926 inoculated seed were planted in the field in order to study the effect of heavy infection under field conditions. The tests of 1925 were unsuccessful, owing to the severe drought which prevailed throughout the summer. Only a few seed germinated.

In 1926, spore suspensions of three cultures were used for inoculating two rows of delinted and sterilized seed and two rows of undelinted and unsterilized seed for each of the cultures. Seed similarly prepared but uninoculated were planted as checks. The inoculated seed were dried before planting.

Heavy inoculation failed to increase greatly the percentage of infection over that of the seed treatment tests in which the seed were not inoculated.

There was little difference between delinted and undelinted seed; although the checks showed a slightly higher percentage of healthy plants.

One month after planting, 200 plants from each two rows were dug, washed, and examined. The counts are given in table 1.

TABLE 1.—*Results of inoculating delinted, sterilized cotton seed and undelinted, unsterilized seed with three different cultures of Fusarium isolated from diseased cotton*

Culture no.	Seed delinted and sterilized		Seed undelinted and unsterilized	
	Pet. plants healthy	Pet. plants diseased	Pet. plants healthy	Pet. plants diseased
40	42.5	57.5	50.5	49.5
61	34.5	55.5	28.5	71.5
64	29.0	71.0	25.0	75.0
Check	64.0	36.0

Nos. 64 and 61 were single spore isolations of a culture from an initial epidermal lesion, No. 61 from a typical macroconidium and No. 64 from a typical microconidium. No. 40 was also a single spore isolation from a lesion on the epidermis of seedling roots. This culture was found in the pots and bottles to be very pathogenic and was used as a standard of comparison for other cultures.

SOURCES OF INFECTION

Early in these investigations it was concluded that spores borne on the lint of the seed were a common source of infection to young cotton seedlings. Plants from undelinted, unsterilized seed in sterile soil had a high percentage of infection, while there were few or no lesions on the roots of young seedlings from both delinted seed and delinted, surface-sterilized seed. A pink boll rot, which is common during damp rainy weather, was also found to be caused by the fungus attacking the roots. Therefore, conidia are in all probability present on a high percentage of the lint which appears clean.

The mycelium grows in the soil and is undoubtedly a source of infection for otherwise healthy seedlings. The importance of soil infection will be discussed in connection with the work on control.

As a boll rot is caused by the same fungus as that attacking the roots, the possibility of the mycelium being borne within the seed was considered and a number of tests made. Plants grown in sterile soil from both delinted and delinted, surface-sterilized seed indicated that internal seed infection is relatively unimportant. Plating surface-sterilized seed on agar also



FIG. 2. Cotton seedlings, 24 days old, from disinfected seed (check) and from seed inoculated with two strains of *Fusarium moniliforme*

showed that in the average lot of seed this is practically a negligible factor. The fungus may be present in the seed of badly rotted bolls but, owing to the difficulty of surface sterilizing such seed and the great number of organisms associated with rotted bolls, this phase of the problem has not been fully worked out

DESCRIPTION AND IDENTIFICATION OF THE CAUSAL ORGANISM

The following media were employed in the study and identification of the causal organism: steamed bean pods, steamed Irish potato plugs, Irish potato agar (2 and 5 per cent dextrose), oat agar, and steamed cotton stems. The first four media were prepared according to the recommendations of Sherbakoff (4) and the Committee on the Taxonomy of the *Fusaria* (6).

Ovoid-fusoid and ellipsoid microconidia are produced in chains on verticillately branched conidiophores, ternate branching being the rule.

A few macroconidia are usually present in all cultures and in scrapings from diseased roots, but only in very limited numbers. However, the number of macroconidia is a variable character. Transfers from cultures producing few macroconidia, as well as a few original tissue isolations, occasionally yielded them in abundance. Single spore isolations tend to produce few or many macroconidia according to the type of conidium isolated.

Macroconidia when produced in abundance are borne in sporodochia, which vary in color from a light ochraceous salmon to a light vinaceous cinnamon (2). Macroconidia are with few exceptions three-septate.

Neither chlamydospores nor sclerotia were noted in any of the cultures. Dark bodies on potato plugs, which appeared to be sclerotia under the hand lens, were found by microscopic examination to be merely dark masses of mycelium, not true sclerotia.

The color and quantity of mycelium varies to a considerable extent. The most common color is a pale flesh or shell pink (2). A pink growth of mycelium and conidiophores may often be seen on the surface of diseased roots.

On the starchy media, shades as dark as a slate violet are characteristic of a number of cultures which are otherwise of the usual type, capable of infecting cotton seedlings. The substratum on which the violet cultures grow becomes dark in contrast with the lack of coloration in the substratum on which the pink cultures are grown.

A fluffy, moderately profuse growth of mycelium is most common in culture, but in a few instances a very suppressed mycelial growth is characteristic.

The moniliform method of bearing microconidia on verticillately branched conidiophores, the absence of chlamydospores, and the three-septate macroconidia indicated that the fungus was a *Fusarium* of the section Moniliforme (*Liseola* of committee) (6). See Fig. 1. According to the descriptions and keys given by the Committee on the Taxonomy of *Fusaria* (6) and those given by Stevens (5) and comparing the organism with Sheldon's description of *Fusarium moniliforme* (3) it seemed to be identical with the species from corn. Cultures of *Fusarium moniliforme* Sheld., secured through the kindness of Dr. C. D. Sherbakoff, confirmed this conclusion.

METHOD OF ATTACK

Diseased and healthy roots of young cotton plants were prepared and sectioned for microscopical study in order to determine, if possible, the method of attack and the depth of penetration of the mycelium of the fungus. Free-hand sections of the older roots stained with eosin and mounted in glycerine were found satisfactory for studying the more advanced stages.

The mycelium apparently never enters the living cells of the root, as sections have not been obtained showing the mycelium within the cells. A layer, one or two and sometimes three dead cells in depth, may be seen about the periphery of the root. All or only portions of a root surface may



FIG. 3. Cotton seedlings from bottles planted with seed inoculated with *Fusarium* culture No. 40, showing girdled tap roots. Photographed 16 days after planting.

be affected. After the first three or four weeks the roots are usually completely girdled. The dead, collapsed and darkened layer of cells may be seen in both cross and longitudinal sections. Figure 3 shows the effect of the disease on the young roots.

Dwarfing is caused by a dry, and not a soft, root rot. A soft rotted condition has never been observed either in the inoculation tests or in the field unless associated with some other organism. Pratt (1) reports a soft rot produced by *F. moniliforme* Sheld. on the radicals of seedlings grown in germination tests. Such a condition has not been observed in the course of this work.

The mycelium persists throughout the entire season on the primary roots. *Fusarium moniliforme* Sheld. has been isolated from bits of the bark from primary roots as late as October 15. However, the fungus never penetrates deeply into the cortical layer. The spread of the disease to the lateral root and the persistence of the fungus on the primary root dwarf the entire plant so that it makes little growth.

As the root grows and expands, the layer of dead cells cracks and splits and probably nearly all of it becomes loosened. Successive layers beneath each layer removed may become diseased. However, the whole process probably proceeds slowly. In some cases the plants even seem to overcome the disease to a certain extent and produce various degrees of dwarfing. During years of plentiful rainfall, few or no plants are girdled to the extent of causing the formation of a knot at the surface of the ground. In dry years the dead layer of cells becomes hard, is not easily ruptured but binds the root, causing the definite symptoms of girdling evidenced by the constricted root with a swelling directly above.

Pratt (1) reports that she invariably found *F. moniliforme* Sheld. within the tissue of rotted embryos of cotton seed from Queensland. Such seed failed to germinate. Miss Pratt also reports successful inoculation of green bolls and ripe cotton seed in which she was able to duplicate the condition found in the seed from Queensland.

INOCULATION TESTS WITH CULTURES OF *FUSARIUM MONILIFORME* SHELD. FROM OTHER SOURCES

A series of three bottles for each of the three cultures of *F. moniliforme* Sheld., secured from Dr. Sherbakoff, were planted with inoculated seed and placed in the greenhouse in order to test their pathogenicity on the roots of seedling cotton plants. The results are given in table 2.

A large percentage of the plants became infected but not so severely as the plants infected by the strains isolated from cotton. The development of lateral roots was not checked to any great extent and the roots were

not so completely girdled. The mycelium of all cultures grew profusely in the soil.

TABLE 2.—*Results of inoculating cotton seeds with three cultures of Fusarium moniliforme Sheld. from different sources*

Culture no. ^a	Per cent plants infected	Per cent plants healthy
R 53	62.8	37.8
R 57	90.3	10.7
1012,3i	93.5	6.5
Check	0.0	100.0

^a No. R53 was isolated from soil in Central America.

No. R 57 is *F. moniliforme* v. *maius* from soil in Central America.

These two cultures were isolated by Dr. O. Reinking and determined by Dr. Woltenweber.

No. 1012,3i was isolated by Dr. Sherbakoff from germinating corn seed.

CONTROL

In conjunction with the study of the dwarfing disease of cotton, possible measures of control were also considered and numerous disinfectants tested. In view of the disagreeable nature of the standard method of delinting with sulphuric acid and soaking in bichloride of mercury, attention was given to the simpler method of dusting the seed. Forty-five different dusts, including commercial dusts and several combinations prepared in the laboratory, were applied at different rates to the undelinted seed.

Germination tests of all dusted seed were first made. Four hundred seed were used for each test, the effectiveness of each dust was measured by the vigor and readiness with which the seed germinated, the percentage of germination and the comparative freedom from mold and rot of the radicles. Comparisons were made with undelinted checks and with the standard bichloride of mercury treatment. The germinator was kept at 25° to 28° C.

Treatments giving the highest percentage of clean seedlings in the germinator tests were used in dusting seed to be planted in beds in the greenhouse. Germination and freedom from root rots were considered in judging the value of the treatments.

Fifteen treatments including an undelinted and a delinted check and the standard mercuric chloride treatment were selected, on the basis of the greenhouse tests, for trial under field conditions. Field tests have been made in two successive years. The 1925 results were discarded on account of the severe drought. The 1926 trials were more conclusive, but it will be necessary to repeat these under other weather conditions. The planting

season of 1926 was followed by a week of cold wet weather, which delayed germination. The results are summarized in table 3.

TABLE 3.—*The percentages of germination of cotton seed in the field and germinator tests, the percentage of dwarfed plants in the field, and the relative effectiveness of fifteen treatments for the control of Fusarium moniliforme Sheld.*

Disinfectant	Rate per bushel	Per cent dwarfed plants in field	Per cent germination in field	Per cent germination in germinator	Freedom from mold and rot
Semesan No. 13U....	4 oz.	44.5	32.10	75.10 ^a	Clean
Du Pont No. 18.....	4 oz.	59.0	27.85	85.88	Clean
Du Pont No. 12.....	4 oz.	60.5	23.45	85.82	Clean
Mercuric chloride dust No. 1.....	4 oz.	63.0	26.95	84.46	Fairly clean
Mercuric resinate ..	Saturated ^b	59.0	26.25	87.30	Clean
Uspulun dust No. 3	Saturated ^b	42.0	17.25	85.70	Clean
Corona 640.....	4 oz.	58.0	24.10	84.10	Fairly clean
Bayer dust	Saturated ^b	46.5	13.80	82.06	Fairly clean
Mercuric chloride and barium carbonate	4 oz.	55.5	24.40	77.85	Fairly clean
Mercurous chloride ..	4 oz.	56.0	15.30	83.10	Fairly clean
Mercuric chloride dust No. 2.....	4 oz.	65.0	22.05	84.20	Fairly clean
Copper carbonate.....	4 oz.	58.0	16.85	84.37	Fairly clean, injured
Delinted	43.0	15.55
Delinted and soaked 30 min. in 1-1000 mercuric chloride..	38.5	21.25	82.53	Fairly clean
Check—no treatment	51.5	18.90	80.84	Poor

^a Results of only one test, while others are averages of several.

^b All the dust the seed would take up.

The percentage of germination in the field is much lower than that in the germinator. In the germinator, with the exception of Semesan No. 13U and mercuric chloride + barium carbonate, the percentages of germination of treated seed average from 2 to more than 5 per cent higher than in the untreated check. Discarded treatments often gave a high percentage of germination, but in these, as well as the checks, the great number of rotted radicals which were included in the counts disqualified them. The germination percentages for five treatments in the field averaged lower than the check. It is possible that the increase in the others over that of the check may have been due to the beneficial effects of the treatment.

It is possible to disinfect cotton seed with any one of a number of dusts, controlling all lint-borne organisms so that clean vigorous seedlings are secured in the germinator. However, when seed treated in the same manner are planted under field conditions, soil infection becomes a problem. Higher percentages of dwarfed plants were present in the treated than in the check rows. The field in which the 1926 tests were made had been planted to cotton the year previous. The soil was, as a result, probably heavily infested with *F. moniliforme*. Therefore, it is apparently a difficult problem, if not entirely impossible, to control *Fusarium* root rot of cotton by seed treatment.

Some of the dust treatments tested appear to be fully as effective in disinfecting cotton seed as the delinting and soaking in mercuric chloride. Dusts are cheaper and more easily applied. However, further field tests will be necessary before definite conclusions can be drawn as to the comparative and actual value of treatments for cotton seed. The tests will be continued and detailed results reported in a future publication.

SUMMARY

Dwarfed cotton plants affected by a distinct type of dry root rot have been noted in the fields of the Georgia Experiment Station for a period of years.

Fusarium moniliforme Sheld. has been determined as the cause of the dwarfed condition.

Dwarfed plants usually remain small throughout the entire season, the causal organism being present on the roots at all times.

The disease is spread on the lint of cotton seed and by air-borne spores, both being means of infecting clean soil with the fungus.

A pink boll rot is caused by *Fusarium moniliforme* Sheld.

Only the outer epidermal cells of the root are killed by the fungus, the cortex and central cylinder remaining healthy.

Cultures of *Fusarium moniliforme* Sheld. isolated from other sources infected the roots of cotton seedlings.

Due to soil infestation with the fungus the disease was not controlled by the use of seed disinfectants.

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A SCLEROTIUM DISEASE OF LARKSPUR

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INTRODUCTION

A study has been made of a disease of the cultivated larkspurs (*Delphinium* spp.), which has been observed for some years in gardens and nurseries in and near Madison, Wisconsin. During 1925 about twenty per cent of the larkspurs in one commercial nursery near Madison was destroyed by this disease. Different horticultural types of larkspur, however, showed different degrees of susceptibility. *D. hybridum* Steph., which has a tall stout stem, appeared rather resistant, while *D. "belladonna"* (Hort.), which has a short delicate stem, was apparently very susceptible. Specimens of *Delphinium* and also of *Physostegia*, which had been destroyed by the same disease, were received from Fort Atkinson, Wisconsin. A disease apparently identical with this has been previously reported on larkspurs from a number of other localities [New York (4), Pennsylvania (1, 4), Indiana (4), and New Jersey (4)].

At Madison, in 1925, the symptoms of the disease became apparent toward the end of June. The first indications were the discoloration of the lower leaves and the wilting of the young shoots, commonly followed within a few days by the death and drying up of the entire plant. At this time a white or greyish white mycelium was found girdling the roots and crown, and forming sclerotia on them. The fungus was commonly confined to these parts. However, during wet weather when conditions were favorable for fungus development, the mycelium was found upon the stems for a distance of five or six inches above the surface of the ground (Plate XI, A, B, and C). Sections of stems in which the disease had reached various stages of development showed that the fungus attack was superficial. Sometimes the mycelium was intercellular in the cortex, where it commonly disintegrated these parts and pushed out the superficial layers. When the soil was examined, the mycelium was found in association with even the smaller roots and seemed to be propagating saprophytically along the cracks of the soil. The disease, although found as early as June, did not develop severely or become conspicuous until August. The severity varied with the condition of the soil, particularly with moisture, the disease increasing rapidly after rainfall.

¹ This research was done under the direction of Prof. L. R. Jones, whom the writer sincerely thanks for his helpful criticisms and suggestions.

THE ASSOCIATED FUNGUS IN CULTURE

The associated fungus was isolated and studied in pure culture. The characters resembled somewhat those of *Sclerotium rolfsii* Sacc. as described from tomato by Taubenhause (3) and as described by Higgins (2). They were also similar to those of *S. delphinii* Welch from larkspur (4). The study of the associated fungus led the writer to the conclusion that the fungus under consideration was not *S. rolfsii*, but one similar to or identical with *S. delphinii*. It will, therefore, be referred to as *S. delphinii* in this paper.

An examination of a plate culture grown at 28° C. on potato dextrose agar for 5 days (Plate XI, D) showed abundant white mycelium developing radially, mainly in strands. These strands were the result of the branching of the mycelium. The branches usually began their growth at an acute angle with the direction of the main hypha. Subsequently the hyphae grew parallel and tended to hold together in rhizomorph-like strands. The hyphae varied in width from 2 to 10 microns, generally being 4 to 8 microns in diameter. Special feeding branches, which were very slender and generally curved, were produced on the culture medium. Clamp connections occurred in the main hyphae, and anastomosis often took place. The cells of the mycelium usually were multinucleate.

After five days the cultures began to form sclerotia, usually at the edge of the plate. The sclerotia were rather spheroidal, somewhat flattened at the base, and 1 to 5 mm. in diameter. Their color was first white, then yellow or buff, and finally dark red or chocolate. As the sclerotia matured the mycelia collapsed and became inconspicuous (Plate XI, E).

Effect of various kinds of media

A study of the characters of the larkspur organism on different media was made. The comparative development of the fungus on 19 kinds of media is given in table 1. The growth of the mycelium and production of sclerotia varied with the medium. On no medium were spores produced.

On some of the media the development of *S. delphinii* was compared with that of two strains of *S. rolfsii* (Wisconsin stock cultures No. 393 and No. 394). A comparison of the mycelial developments showed no significant differences except that the colony of *S. rolfsii* produced a more dense, cotton-like mass of mycelium than *S. delphinii*. However, distinction appeared in size, shape, and number of sclerotia. The sclerotia of *S. delphinii* were conspicuously larger than those of the two strains of *S. rolfsii*. On potato dextrose agar the sclerotia of *S. delphinii* varied from 1 to 5 mm., commonly 2.5 to 3.5 mm. in diameter, while the sclerotia of *S. rolfsii* (No. 394) varied from 0.9 to 2.5 mm., commonly 1.4 to 1.7 mm. in diameter, and those of the

other strain (No. 393) varied from 0.3 to 1.5, commonly 0.7 to 0.9 mm. in diameter.

TABLE 1.—Comparative development of cultures of *Sclerotium delphinii* on different media, grown at 28° C. except as noted

Medium	Mycelial growth after 4 days ^a	Sclerotial formation after 10 days ^a	Diameter of sclerotia in mm.
Potato dextrose agar	+++	+++	1-5
Synthetic agar	+	+	1-2
Soil extract agar	++	0	—
Bouillon agar	+	0	—
Bouillon dextrose agar	+++	+	1-2
Prune agar	+++	+++	1-3
Oatmeal agar	+++	+++	1-3
Cornmeal agar	+++	+++	1-3
Larkspur decoction agar	++	+	1-3
Iris decoction agar	+++	+++	1-3
Horse dung	+++	+++	2-6
Boiled rice	+++	+++	2-15
Boiled potato	+++	+++	2-15
Raw potato	+++	+++	2-6
Larkspur stem	+++	+++	1-3
Water melon ^b	+++	+++	2-4
Tomato fruit ^b	+++	+++	1-3
Pepper fruit ^b	+++	+++	1-3
Citrus fruit ^b	++	+++	1-3

^a +++, abundant; ++, moderate; +, scant growth; 0, no formation of sclerotia.

^b Cultures grown at approximately 20° C.

There was also a striking difference in the number of sclerotia produced by *S. delphinii* and by *S. rolfsii*; the larkspur fungus produced fewer sclerotia (table 2). It was found, however, that the size and number of sclerotia of both species could be made to vary with the amount of culture medium used. In determining this relation, petri dishes 9 cm. in diameter were used, in some of which 10 cc. and in others 20 cc. of potato dextrose agar was poured. A greater number of sclerotia of somewhat larger size was produced on the deeper medium. There was, however, always a marked difference between those of *S. delphinii* and *S. rolfsii*.

The sclerotia of *S. delphinii* were variable in shape, usually spherical, slightly flattened or even concave on the under side, particularly when they were large. The surfaces of the sclerotia were very often pitted. In color the sclerotia were dark red or chocolate, conspicuously redder than those of *S. rolfsii* of which both strains were dark brown.

TABLE 2.—*Relative number of sclerotia produced in plate cultures of Sclerotium delphinii, and Sclerotium rolfsii grown at 27° C. for 10 days*

Medium	Amount (cc.)	Number of sclerotia		
		<i>S. delphinii</i>	<i>S. rolfsii</i> (No. 393)	<i>S. rolfsii</i> (No. 394)
Potato dextrose agar	10	33	226	238
do	20	69	554	276

Effect of temperature

The effect of temperature on the growth of *S. delphinii* in culture was studied in comparison with that on two strains of *S. rolfsii*. The results are given in table 3. Development after one month was as follows: at 5° C. none of the fungi had grown; at 10° C. *S. delphinii* had developed a very scant mycelium, while neither strain of *S. rolfsii* showed any growth; at 14° C. *S. delphinii* had produced mature sclerotia, *S. rolfsii* No. 394 white abortive sclerotia, and *S. rolfsii* No. 393 an abundant white mycelium but no sclerotia; at 18° C. *S. delphinii* and *S. rolfsii* No. 394 had formed mature sclerotia, but *S. rolfsii* No. 393 an abundant white mycelium without sclerotia; at 21° C. *S. rolfsii* No. 393 had produced small sclerotia here and there, and the other two cultures mature sclerotia. Above this temperature all the fungi produced mature sclerotia. The result of this experiment, therefore, showed that *S. delphinii* can grow at a rather lower temperature than *S. rolfsii*. The optimum temperature for *S. delphinii* appeared to be from 28° to 30° C.

TABLE 3.—*Average diameters in millimeters of two colonies each of Sclerotium delphinii and Sclerotium rolfsii grown at different temperatures for four days on potato dextrose agar*

Fungi	Temperature in degrees C.							
	30.5	28	24	21	18	14	10	5
<i>S. delphinii</i>	71.5	71.5	40.5	35.0	4.5	3.0	0	0
<i>S. rolfsii</i> No. 393	64.0	54.0	30.0	21.0	6.5	2.0	0	0
<i>S. rolfsii</i> No. 394	72.0	70.0	31.0	22.5	2.0	2.0	0	0

PATHOGENICITY OF THE FUNGUS

Both gross and microscopic examinations indicated that the disease was caused by the associated fungus (*Sclerotium delphinii*). In order to deter-

mine the relations, inoculations were made on several species of plants with pure cultures of the organism.

In the first experiment three healthy larkspur plants, which had grown for two years in the nursery, were transplanted into pots and ten days later, on July 7, inoculated as follows: the soil was dug from about the stem for a depth of 5 mm. and mycelium from a potato agar culture of the fungus was placed next to the stem and covered with soil. The plants were kept in the moist inoculation chamber for 24 hours and then removed to the greenhouse. The mycelium spread a little, but soon formed sclerotia and no infection occurred. The following facts may explain the negative results: (a) The period in the inoculation chamber was too short; (b) the greenhouse was dry and the pots were watered but once a day. These conditions may have made the soil too dry for the fungus to develop and produce infection.

In the second experiment, on August 13, inoculations as listed in table 4 were made. The inoculation method was the same as in the first experiment except that the plants were kept in the moist inoculation chamber for two days and were then watered twice each day.

TABLE 4.—Results, after 14 days, of inoculating various plants with *Sclerotium delphinii*

Plants inoculated	Age of plants	Number of plants		Number of plants	
		Inoculated	Infected	Uninoculated	Infected
<i>Delphinium hybridum</i> ...	2 years	2	2 ^a	1	0
<i>D. "bella-donna"</i>	6 months	3	2	2	0
Bean	29 days	7	0	3	0
Cucumber	do	7	1	2	0
Tomato	do	7	0	3	0

^a One plant killed. The other plant lost the old shoots but eventually grew new ones.

The inoculations made in the third experiment, on August 29, are shown in table 5. In this experiment the plants were kept in the moist inoculation chamber for 4 days. The subsequent temperature also was somewhat higher than in the preceding experiments, ranging from 25° to 30° C.

The results of these experiments, as given in tables 4 and 5, showed that the fungus was parasitic on the larkspur, and that it might also attack melon, cucumber, and rice if the conditions were favorable for infection. The symptoms on these inoculated larkspurs were essentially like those of the original disease. On the cucumber the fungus invaded and rotted the

stem at the ground line, the decayed parts collapsing and turning a brownish color.

TABLE 5.—Results, after 10 days at 25–30° C., of inoculating various plants with *Sclerotium delphinii*

Plants inoculated	Age of plants	Number of plants		Number of plants	
		Inoculated	Infected	Uninoculated	Infected
<i>Delphinium</i>					
“belladonna” ...	6 months	2	2	0	—
<i>D. ajacis</i>	2 months	10	2	2	0
Bean	45 days	10	0	3	0
Cucumber	do	5	2	1	0
Tomato	do	10	0	2	0
Melon	36 days	3	1	1	0
Squash	do	2	0	1	0
Rice	45 days	3	1	1	0

SUMMARY

1. A disease of larkspur is reported from Madison, Wisconsin, and vicinity. Different varieties of larkspur have different degrees of susceptibility to the disease. Cultural studies show it to be caused by a fungus similar to or identical with *Sclerotium delphinii*.

2. The mycelial characters of *S. delphinii* and *S. rolfsii* are similar. The sclerotial characters of these species, however, differ. The sclerotia produced by *S. delphinii* are fewer in number, conspicuously larger, usually more flattened, and redder in color than those produced by *S. rolfsii*. Also, *S. delphinii* is able to develop at a somewhat lower temperature than *S. rolfsii*.

3. The production of spores has never been observed even though the fungus was grown on 19 different media.

4. The depth of the medium influences the size and number of sclerotia produced by the fungus. They are smaller and fewer in number on the thinner substratum.

5. The development of *S. delphinii* is affected by temperature, making but scant growth at 10° C. and apparent optimum growth at 28° to 30° C.

6. The fungus has been found pathogenic to larkspur and, to a lesser degree, to melon, cucumber, and rice.

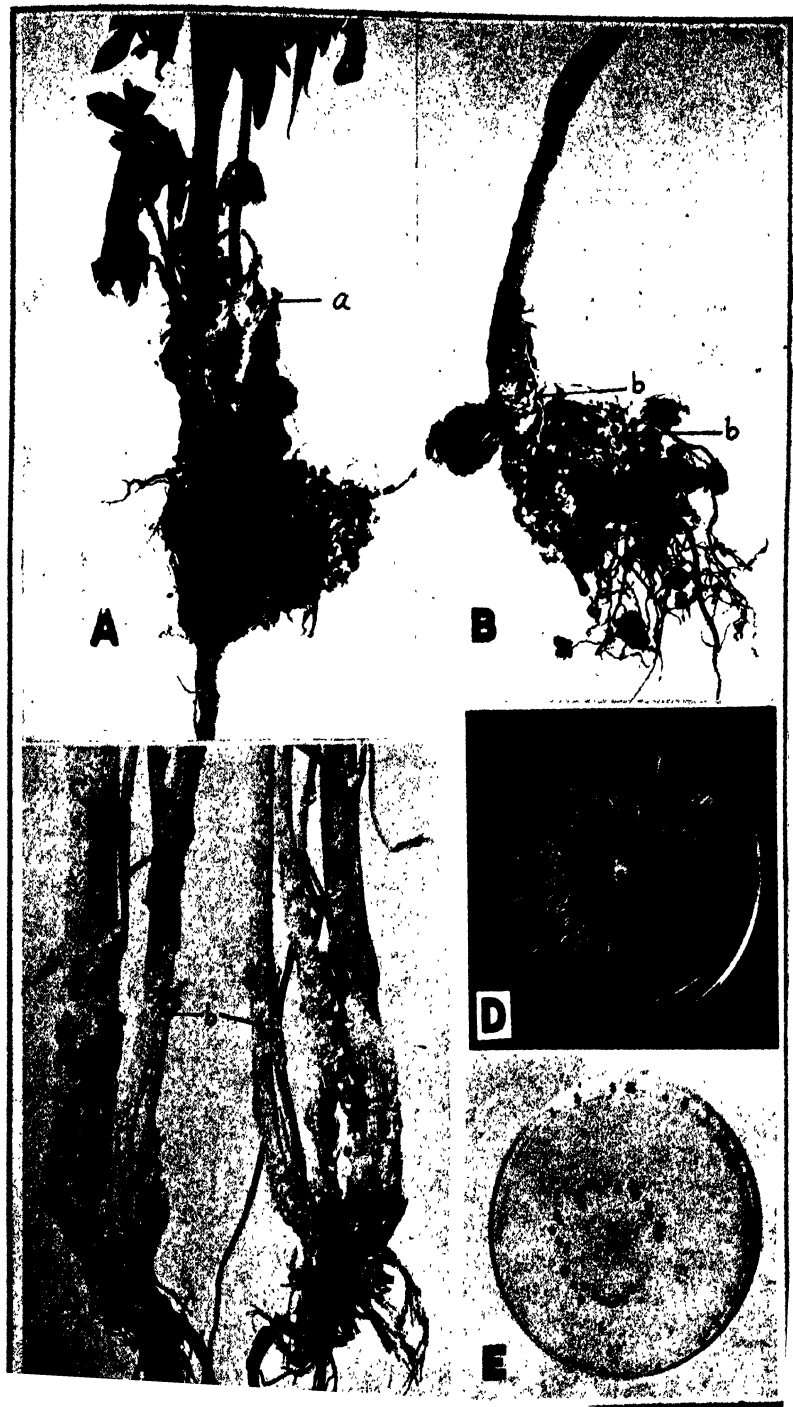
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EXPLANATION OF PLATE XI

- A. Larkspur newly infected by *Sclerotium delphinii*. White mycelium girdling the crown is seen at *a*.
- B. Symptoms on larkspur a few days later than A. There are numerous new sclerotia on the roots, a few of which are indicated by the arrows marked *b*.
- C. Typical symptoms of larkspur attacked by *S. delphinii*. The roots are almost entirely rotted, and the stem is dying from the base upward. Some sclerotia are seen on the stem at *b*.
- D. Development of mycelium on potato dextrose agar after 3 days at 28° C.
- E. Formation of sclerotia on potato dextrose agar after 10 days at 28° C.



PHYSIOLOGIC SPECIALIZATION IN *TILLETIA LEVIS* AND *TILLETIA TRITICI*¹

H. A. RODENHISER AND E. C. STAKMAN²

INTRODUCTION

With the increased attention during the past few years to the development of disease-resistant varieties of crop plants, the investigation of the possible rôle of physiologic specialization of the pathogenes involved assumes additional importance. While most of the cereal smut fungi can be prevented fairly well by seed treatment, the method is not always completely effective. In any case, it is desirable to use disease-resistant varieties if they are available. One of the important factors in determining the value of disease-resistant varieties is the degree of parasitic specialization, mobility, and genetic constancy of the pathogene. It is known that there are different physiologic forms of some pathogenes in different regions, and that different forms may be present in the same region in different years (6).

Reed (4), Faris (2), Stakman and Christensen (8), Christensen and Stakman (1), Rodenhiser (5), and Tisdale and Johnston (9) have shown that certain of the smut fungi comprise distinct physiologic forms. It seemed quite likely that the same might be true of *Tilletia levis* Kühn and *Tilletia tritici* (Beij.) Wint., especially since Faris (3) obtained some preliminary evidence of differences in pathogenicity.

In the hard red spring wheat region of the United States, bunt of wheat has not been very destructive during the past ten years. The principal reason seems to be that Marquis and the durum wheats, which are grown most commonly, are resistant. But during the summer of 1925 there was an unusual outbreak of bunt, caused principally, in Minnesota at least, by *T. levis*, although *T. tritici* also was found to some extent. The natural supposition would be either that weather and soil conditions were unusually favorable for the development of bunt, or that there might have been un-

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² The writers are indebted to the following persons for bunt material: Dr. F. N. Briggs, California; Dr. F. D. Heald, Washington; Dr. B. Husz, Hungary; Dr. E. Pantanelli, Italy; Dr. I. Jorstad, Norway; Dr. T. Lindfors, Sweden; Dr. T. Fahmy, Egypt; Dr. G. H. Cunningham, New Zealand; and Mr. I. I. Connors, Canada.

usually virulent strains of the pathogene. Therefore the writers attempted to ascertain whether there actually are distinct physiologic forms of *T. levis* and *T. tritici*.

MATERIALS AND METHODS

Five collections of *T. levis* and seven of *T. tritici* were obtained in 1925, from the places indicated in tables 1 and 2.

In the spring of 1925 Kota wheat, C. I. 5878,³ was inoculated with each collection of smut except those from California and Washington, which had not yet been obtained. The smutted heads which resulted were then picked and kept under the same conditions until the spring of 1926. Thus the spores of all of the collections, with the exceptions mentioned, were produced and kept under similar conditions.

Mindum, C. I. 5296, a durum wheat; Einkorn, C. I. 2433; Marquis, C. I. 3641, and Kota, C. I. 5878, both hard red spring wheats, were inoculated with chlamydospores of each collection of smut. Previous to inoculation, the seed was treated by Jensen's modified hot water method. When thoroughly dry, enough seed for duplicate rod rows was inoculated with powdered inoculum from each collection, at the rate of 0.5 grams to 100 grams of seed, and all seed was sown on the same day in duplicate, systematically distributed rod rows. As there was very little difference in the percentage of smut in the duplicate rows, only the averages are recorded.

With one exception, the percentages of infection given in tables 1 and 2 are based on counts of 800 heads of Einkorn, 800 of Marquis, and 1,000 of Kota. The counts on the varieties inoculated with the Egyptian collection are based on counts of 400 heads of Einkorn, 400 of Marquis, and 500 of Kota. The percentages of partially and completely smutted heads of Einkorn and of Marquis were recorded separately. The number of partially smutted heads of Kota was not determined, because there was very little partial smutting. So little smut developed in Mindum that the results are not recorded.

RESULTS

The results of the tests are given in tables 1 to 3 inclusive. The results with Einkorn and Marquis are considered more significant than those with Kota, because the two former usually are so resistant that one would not expect much variation in the percentage of infection. These varieties have been grown at University Farm, St. Paul, for seven years, from artificially smutted seed, and there always has been a high degree of correlation be-

³ C. I. = accession number of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

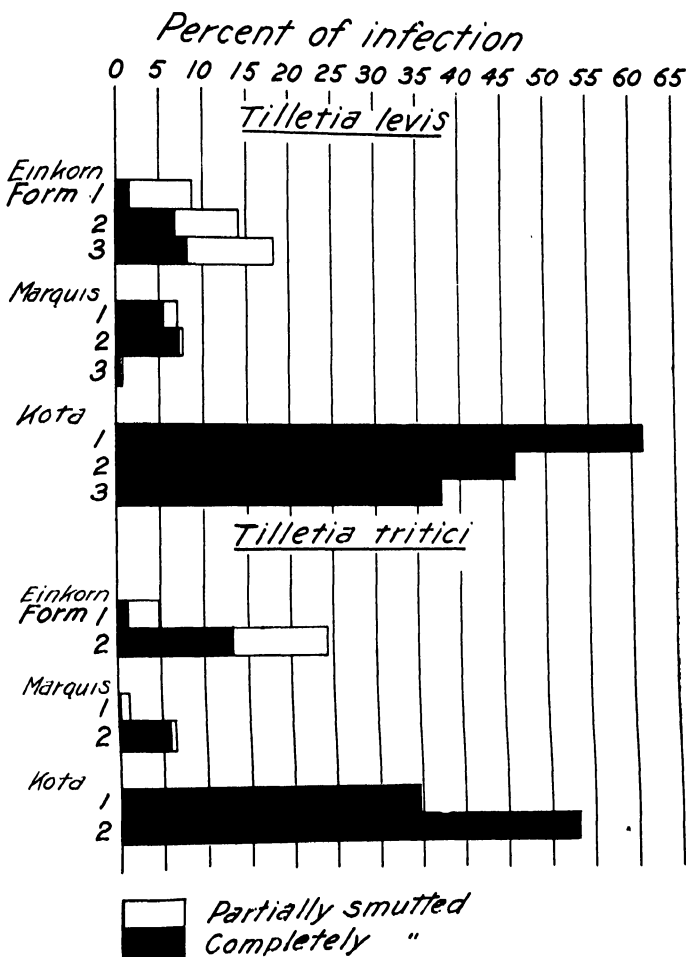


FIG. 1.—The percentages of infection in Einkorn, Marquis, and Kota inoculated with three physiologic forms of *Tilletia levis* and two of *T. tritici*.

tween the percentages of bunt in different years, as well as in replicated rod rows in the same year.

It is evident from figure 1 and tables 1 and 2 that there are sufficiently great differences in the virulence of several collections of smut to justify the conclusion that there are physiologic forms. In table 3 is indicated the relative susceptibility of each variety to each of these forms.

It seems clear from table 3 that there are at least three physiologic forms of *T. levis*. The two collections from Hungary were so nearly alike that they are considered to be identical and are designated as form 1. The collection from Minnesota behaved much like form 1 on Marquis and Kota,

TABLE 1.—The percentage of smutted heads in Marquis and Kota wheats and Einkorn inoculated artificially with five collections of *Tilletia levis* at University Farm, St. Paul, Minn., in 1926

Source of inoculum	Germination of chlamydo-spores in per cent	Percentage of smutted heads					
		Einkorn		Marquis		Kota	
		Partial	Complete	Total	Partial	Complete	Total
Minnesota	65	8.4	6.7	15.1	0.4	7.1	7.5
Hungary ^a	80	12.7	1.9	14.6	0.9	4.6	5.5
Hungary ^b	75	7.4 ^c	1.5	8.9	1.7	5.3	7.0
Italy	80	13.4	7.3	20.7	1.2 ^c	0.2	1.4
Egypt	90	9.8	8.3	18.1	0.3 ^c	0.3	0.6
Check (uninoculated)	0.0	0.0	0.0	0.0	0.0	0.0
		Total complete		Total		Total complete	
		64.3		7.5		61.7	
		61.1		5.5		61.1	
		56.7		1.4		56.7	
		37.6		0.6		37.6	
		0.0		0.0		0.0	

^a Hatvan.

^b Debreczen.

^c Individual heads lightly smutted. Only a few smutted, or partially smutted, kernels in each head.

TABLE 2.—The percentage of smutted heads in Marquis and Kota wheats and Einkorn inoculated artificially with seven collections of *Tilletia tritici* at University Farm, St. Paul, Minn., in 1926

Source of inoculum	Germination of chlamydospores in per cent	Percentage of smutted heads						
		Einkorn		Marquis			Kota	
		Partial	Complete	Total	Partial	Complete	Total	Total complete
New Zealand	80	3.7 ^b	1.1	4.8	1.0	0.3	1.3	34.5
Hungary ^a	65	9.5 ^c	9.7	19.2	0.4	2.7	3.1	50.4
Norway	70	11.1	13.2	24.3	0.6	5.9	6.5	53.4
Sweden	80	9.9	1.9	11.8	1.8 ^b	0.7	2.5	58.0
Manitoba	80	5.8	6.4	12.2	1.0	4.2	5.2	37.5
California	80	10.0 ^c	13.7	23.7	1.5	0.5	2.0	40.0
Washington	65	4.0	6.5	10.5	1.4	0.4	1.8	28.5
Check								
(Uninoculated	...	0.0	0.0	0.0	0.0	0.0	0.0	0.0
• Hatvan.							*	

^a Individual heads lightly smutted.

^b Individual heads almost completely smutted.

^c Individual heads almost completely smutted.

but was considerably more virulent on Einkorn and therefore is considered as form 2. The collection from Egypt was slightly more virulent on Einkorn than the other two, decidedly less virulent on Marquis, and considerably less virulent on Kota. Therefore it is designated as form 3. The collection from Italy was very similar to the one from Egypt, although apparently less virulent on Marquis and Kota. It may be a fourth form, but, until further tests are made, it is not so considered.

TABLE 3.—The relative susceptibility, of Einkorn, Marquis and Kota to four collections of *Tilletia levis* and to three of *T. tritici*

Source of inoculum	Relative susceptibility ^c			Form number
	Einkorn	Marquis	Kota	
<i>Tilletia levis</i>				
Hungary ^a	R	R	S	1
Hungary ^b	R	M R	S	1
Minnesota	M R	M R	S	2
Egypt	M R	V R	M S	3
<i>Tilletia tritici</i>				
New Zealand	V R	V R	M S	1
Norway	M R	M R	S	2

^a Hatvan.

^b Debreizen.

^c V R = very resistant; M R = moderately resistant; M S = moderately susceptible; S = susceptible.

One could conclude that there are at least five distinct forms of *T. tritici* (table 2), but no final conclusions are based on the results obtained with the collections from Washington and California, because the inoculum was not produced and stored under the same conditions as those for the other collections. Nevertheless there are at least two forms: form 1, from New Zealand and form 2, from Norway. The New Zealand collection was consistently less virulent than that from Norway and the differences are so great that one is forced to the conclusion that they represent two distinct forms. For the present, the collection from Hungary is considered to be form 2. The collections from Manitoba and Sweden are similar to forms 2 and 1 respectively on Einkorn and Marquis, but both collections differ somewhat in their effect on Kota and it is probable that they can be differentiated more clearly on other varieties of wheat.

SUMMARY

1. *Tilletia levis* and *T. tritici* both comprise distinct physiologic forms which can be recognized by the degree of their virulence on Kota and Marquis wheats and on Einkorn.

2. Collections of *T. levis* were obtained from Minnesota, Italy, Egypt, and two localities in Hungary. There were at least three physiologic forms in these collections: one from Minnesota, one from Hungary, and one from Egypt. The Italian collection possibly may represent still another form.

3. Collections of *T. tritici* were obtained from New Zealand, Hungary, Norway, Sweden, Canada (Manitoba), and from Minnesota, California, and Washington in the United States. Two forms can be recognized readily: a virulent one from Norway, and a relatively weak one from New Zealand. There is evidence that there are still other forms.

4. It seems likely that a considerable number of forms both of *T. levis* and *T. tritici* can be distinguished if the proper differential hosts are used.

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SECOND PROGRESS REPORT ON BUNCHY-TOP OF ABACÁ, OR MANILA HEMP

GERARDO OFFIMARIA OCFEMIA

In the abacá plot of the Department of Agronomy of the College of Agriculture at Los Baños, Laguna Province, Philippine Islands, in specimens collected from Cavite Province, and in transmission experiments of bunchy-top using *Pentalonia nigronervosa* under controlled conditions, it has been noted that in advanced stages of the disease "heart rot" sometimes sets in. This observation is of special significance, because of the attention which heart rot has attracted. According to Mr. Melanio R. Calinisan, who is working on the relation of nematode (*Heterodera radiculicola*) to bunchy-top of abacá, the abacá farmers of Cavite Province believe that the heart rot is more important than the bunchy-top. In a conference with Mr. Felicísimo B. Serrano, Assistant Plant Pathologist of the Philippine Bureau of Agriculture in Manila, who has been working on the heart rot of abacá since 1920, it was learned that Mr. H. Atherton Lee, formerly Mycologist of the Philippine Bureau of Science in Manila, considered heart rot as the more destructive of the two abacá diseases. Lee and Serrano¹ reported having isolated a *Fusarium* closely resembling *F. cubense* from cases of heart rot of abacá. These authors claim that they obtained positive results from inoculation experiments using *F. cubense* for the production of abacá heart rot. It is not clear, however, how this vascular *Fusarium* of the banana could produce heart rot on abacá.

In aphid-transmission experiments of bunchy-top of abacá under controlled conditions, it has been noted that the first symptom of the disease is the appearance of yellowish white, indefinite chlorotic areas on the margin of the youngest leaf. The green parts of the leaf blade on each side of the midrib are darker green than the leaves of normal plants. The leaves produced are smaller and show a tendency to curl up along the margin. As the curling of the leaf margin is a more characteristic symptom of the virus form of bunchy-top of abacá than the bunching of the leaves, the disease might better be known as "curly-top." The presence of the greenish yellow or yellowish white areas and its transmissibility by aphids might group the disease with the mosaics or transmissible chloroses. The chlorotic areas

¹ Lee, H. Atherton, and F. B. Serrano. Banana wilt of the Manila hemp plant. *Phytopath.* 13: 253-256. 1923.

are retarded in their normal growth, and as a result an affected leaf has a tendency to tear along the margin. Very often delicate, thin, transparent, membrane-like areas of different shapes and sizes are present on the chlorotic portions of the youngest leaf. These membrane like areas are visible before the leaf unfolds or immediately after (Fig 1, a). When the transparent membrane-like areas are present on the greater portion of the unexpanded



FIG 1. (a) A young leaf of abacá, C A 4293 Itom, affected with bunchy top from aphid transmission experiments showing the membrane like areas (m) along the margin of the leaf. The membrane like tissues on the upper part of the leaf were torn off.

(b) At the left is shown the youngest leaf of a healthy abacá seedling, C A. 4293 Itom. At the right is the heart of a bunchy top infected abacá seedling from aphid-transmission experiments showing the browning of the youngest leaf (r) and the beginning of the rotting of the brown tissue.

All photographs were taken by the Photographic Laboratory of the Bureau of Science at Manila, P I, two months after inoculation

youngest leaf, browning follows, and if weather conditions are favorable rotting sets in (Fig. 1, b), starting from the top and working downwards. In this type of heart rot, bacteria are present in great abundance and hasten the rapid decay of the soft tissues of the heart. This type of heart rot is probably the same as Reinking's bacterial heart rot of abacá.²

Although it is not claimed that all heart rots of abacá are secondary diseases, it seems that, in bunchy-top infected districts at least, many of the heart rot cases are probably the final stages of bunchy-top.

In results thus far obtained, it has been noted that the abundance of nematode galls in the roots of abacá, the rotting of roots due to various soil fungi, and close planting may induce bunching of the leaves resembling that of the aphid-transmissible bunchy-top. In bunchy-top brought about by any of these three conditions, however, the chlorotic areas on the leaves, the diminution of the size of the leaf, the curling of the margin, and other malformations are not shown. Further work is in progress.

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² Reinking, Otto A. Philippine economic-plant diseases. Philippine Jour. Science Sec. A. 13: 165-274. 1918.

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PREVENTION OF SEEDLING DISEASES OF SUGAR BEETS

G. H. COONS AND DEWEY STEWART

Each year the root diseases of sugar beets (*Beta vulgaris* L.) cause considerable loss. Practically every state where sugar beets are grown has submitted to the Plant Disease Survey of the U. S. Department of Agriculture reports of damage from damping-off and other forms of root disease. These losses range from a trace to 20–25 per cent of the crop, but the occurrence of the trouble is sporadic and seems correlated with unfavorable growing conditions in the spring. Due to variation in severity from year to year and due to greater emphasis given in the literature to the other more striking diseases of the sugar beet, losses from this source have largely been overlooked. The common cause of failure to get a stand in fungous attack, which gives rise to the so-called “seedling diseases” of sugar beets. A general discussion of the nature of these diseases and their importance under Michigan conditions has been given by Coons (1), who emphasizes the significance of the diseases contracted in the seedling stage in the general root rot development of half-grown and mature beets.

The actual importance of seedling diseases can best be judged by taking into consideration the enormous differences in stands which are obtained in various fields planted with the same seed stocks, under fairly normal conditions. These fields range from full stands to practical failures of stands. The majority of fields show about 75 per cent of the possible stand, and there are many fields on the border line of profit and loss. In Michigan, where this study was made, the seedling diseases and the resultant root-rot constitute the most important sugar beet disease problem. The greatest losses occur on the heavier and the muck soils, and especially on those with poor natural drainage.

Sugar beets are commonly grown in a four-year rotation, as experience has taught the hazard of following beets with beets. In many sections, the use of beets after clover or alfalfa is a common practice. Observations of the writers, extending over many seasons, seem to indicate that, in general, better stands of beets are obtained when beets follow corn instead of legumes.

Indeed, in one section of Michigan, the farmers within a decade have adopted in large measure the latter practice. Clover, vetch, and alfalfa roots show many *Rhizoctonia* lesions and it is the writers' belief that these crops, as well as potatoes, serve to intensify *Rhizoctonia* infestation, while with grain cropping the *Rhizoctonia* infestation is lessened. In Michigan, the sugar beet crop is planted over a period ranging from April 20 to June 20. The standard practice is followed of sowing heavily (15 to 18 pounds per acre) in drill rows, 20 to 24 inches apart, and later thinning to approximately 12 inches in the row. The heavy seeding is an attempt to allow for the very uncertain sprouting of the seed and the heavy reduction of stand by disease. It is obvious that the crowding in the row and the weakening of the plants greatly favors the development of diseases of this type. Cultivation is advised as soon as rows can be followed. The young plants are allowed to grow with no attempt at thinning until decision can be made as to the quality of the stand. If soil and weather conditions have been favorable and cultivation timely, a stand profitable to work generally results. A stand giving promise of at least 7 tons per acre is necessary because of the heavy labor charges involved in the crop. On the other hand, heavy rains, or rainy periods of long duration, low temperature, poor seed, and insufficient drainage all operate to produce poor stands. In such cases, the fields are reworked and either replanted to beets or to some other crop. There have been seasons when as much as 25 per cent of the acreage planted failed to show a profitable stand and was replanted or abandoned. It is obvious that the matter of a good stand is fundamental to success with the crop, and the tests reported in this paper are concerned with seed treatment as affecting stand.

PREVIOUS WORK ON SEEDLING DISEASES OF SUGAR BEETS

Root disease, black rot, or black leg has long been known as a sugar beet disease problem. In the old literature, insects and soil factors were believed responsible. Hellriegel (7) first called attention to the fungous and bacterial factors involved when by a 20-hour soaking of the seed in 1 per cent carbolic acid solution he lessened the root-rot. He attributed this effect to the disinfection of the seed ball. The work of Wimmer (16), Wilfarth (17), and Karlson (9) substantiated this viewpoint. Frank (6) and Krüger (10) later demonstrated the seriousness of *Phoma betae*, but the tendency of nearly all of the older research work was to look upon root disease as an indication of some defect in cultural conditions rather than a parasitic relation influenced by environmental conditions.

Duggar and Stewart (4), in 1901, proved that *Corticium vagum* B. and C. *solani* Burt. (called by them *Rhizoctonia*) was capable of killing sugar beet

seedlings. Pammel (11), Selby (15), and Duggar (3) had previously reported *Rhizoctonia* as causing root-rot in fields of mature beets.

European literature for many years has had numerous, more or less intensive studies on sugar-beet root diseases called "Wurzelbrand." In a series of reports between 1906 and 1911, Peters and his associates (12) went over the voluminous literature and from this and their own experiments concluded that *Pythium debaryanum* Hesse, *Phoma betae* (Oud.) Fr., *Aphanomyces laevis* de By. were the organisms concerned in the production of seedling diseases of sugar beets in Germany. The German investigators were unable to produce damping-off with *Rhizoctonia violacea* Tul.

In 1915, Edson (5) working at Madison, Wisconsin, found that *Phoma betae*, *Pythium debaryanum* and *Rhizoctonia* spp. as well as an organism which he later named *Rheosporangium aphanidermatum*,¹ were the principal organisms concerned in the seedling diseases of sugar beets in the United States. Each organism produced a high percentage of diseased plants when introduced into the seed bed. Edson also found *Phoma betae* present in all lots of seed balls from Europe or America examined, thus confirming the previous results of Peters.

Although *Phoma* is constantly being introduced into sugar beet fields, Pool and McKay (13) have shown that it does not live from year to year in the soil except on fragments of sugar beet tissues. However *Rhizoctonia*, and the Phycomycetes, *Pythium debaryanum*, *Aphanomyces laevis*, and *Pythium aphanidermatum*, are common soil organisms (Jensen, 8; Drechsler, 2) widely distributed in nature.

The problem of controlling sugar beet seedling diseases is, therefore, concerned with the seed-borne fungus, *Phoma betae*, and the numerous soil inhabiting fungi capable of attacking beets. It is obvious that since non-infested soil cannot be found for use with disinfected seed, seed treatment at best can be only partially effective.

Besides this difficulty, it has been found by several experimenters that treatment of seed to eliminate *Phoma betae* was not possible. Edson (5), in an attempt to free seeds from *Phoma betae*, tried three different treatments: (a) strong solution of hydrochloric acid, (b) concentrated sulphuric acid for one hour, and (c) two per cent formaldehyde solution. Each treatment was used for periods sufficient to injure the seedling without materially reducing the subsequent development of *Phoma*.

However, Peters' method of pasteurization at 60° C. for ten minutes on two successive days gave one *Phoma*-diseased plant in about three or four hundred. Edson (5, p. 138) states that this method is not practical for field use, as the germination is reduced. In 1924, Miss Rumbold (14)

¹ Now known as *Pythium aphanidermatum* (Edson) Fitzp.

reported favorable results in sugar beet seed disinfection using formaldehyde and steam in a sort of combination pasteurization and disinfection system. This method has not come into general use.

DESCRIPTION OF SEEDLING DISEASES

The diseases of sugar beet seedlings considered in this investigation are those commonly known as black root, "root disease," and damping-off. These names, more or less descriptive of the diseased seedlings, are used loosely in the literature to apply to death of seedlings from one cause or another. By field examination it is not ordinarily possible to assign the root rot to a particular organism, but culture work is necessary for determination of the causal factor. In one type of disease the seedling shows a browning and blackening of the hypocotyl and root. The discoloration usually shows above the surface of the ground before the seedling topples over. The killing may be fairly rapid or take place so slowly that the seedling seems almost ready to outgrow the disease. Plants are frequently found with hypocotyl completely blackened as far as the cotyledon, which remains turgid and green. Examination of such plants shows the vascular region as the only part not affected. The general impression one has from the examination of such a seedling is that the attack has been made by a moderately rapidly growing organism, which produces a dry type of decay.

Contrasted with this type of disease is one in which rapid wilting occurs, usually unaccompanied by marked discoloration. Such plants have a brown decayed region in the root, and the central vascular region is discolored far in advance of the external lesions. The lesions have a water-soaked appearance as compared with the dry, black lesions characteristic of the first type of attack. Twenty-four hours after the first indications of wilting the seedling is almost completely decayed.

In a third type of seedling disease, which is not so distinctive in appearance as the others, the color of the young leaves gives the first indication of disease. The leaves may be merely deeper green in color, but occasionally they are blue green. Associated with this sign, one finds often a lemon-yellow color of the stem. The seedlings grow slowly and, in general, show evidence of malnutrition. On removing the seedling from the soil, the tap-root is found decayed at the tip and the rootlets above the decayed region are developing, apparently attempting to replace the primary root. No doubt, many of the seedlings thus affected develop into marketable mature beets, but of poor type.

It is hazardous to assign particular organisms to these three disease aspects. As has been said, the beet seedling shows but slight distinctiveness in reaction to the various organisms, and many of the infections are un-

doubtedly mixed infections. The organism obtained by culturing a diseased seedling, especially if this is done tardily, may not be the primary organism concerned. Furthermore, the type of medium used for plating influences strongly the fungous growth which develops from the decayed seedlings, and may even determine the flora which develops.

Generally our results have shown that *Phoma betae* was chiefly associated with the first type of disease, *Pythium* spp. with the second type, and *Rhizoctonia* spp. with the third type.

Mention should also be made of a type of injury often overlooked but exceedingly common. This is the rotting or damping-off of the seedling almost immediately upon its emergence from the seed ball. Such seed balls behave as if non-viable, while in reality the sprout germinates and the little seedling is killed immediately upon emergence. Under wet soil conditions this may be the most common form of attack. From such material *Pythium* spp. have commonly been isolated, but *Phoma betae* is doubtless also responsible.

SEED INFESTATION BY PHOMA

As has been indicated, many workers have demonstrated the presence of *Phoma betae* in commercial sugar beet seed. We were desirous of determining whether this fungus could be eliminated by more care in seed production. Investigation was made using samples of seed from the special stocks of seed produced in the sugar beet breeding work of Mr. E. E. Down, of the U. S. Department of Agriculture. These samples were from isolated breeding plots in the vicinity of Lansing, and the seed had been grown from carefully selected, entirely sound roots which had been carried through the winter under excellent conditions. The seeds were hand-cleaned and represented seed grown with as much care as possible.

Fifty seed balls each of 15 samples were planted in sterile sand in moist chambers. As the seedlings began to die following germination, examination for *Phoma* was made. *Phoma betae* was found in 10 samples of the 15.

This test indicates the very high degree of infestation by *Phoma betae* which exists in seed stocks, since even the careful handling, as in seed breeding work, failed to eliminate it.

METHODS

In preliminary work, the sugar beet seed balls were treated with various disinfectants in both liquid and dry form. Large moist chambers with absorbent paper were used, but it was found that the seedlings soon became covered with *Alternarias* and *Mucors*. When certain chemicals such as mercury bichloride were used, these leached from the seeds and showed

harmful effects on the roots. When moist sand was substituted for the paper, the seedlings died after a few days, due to excessive humidity within the chamber. The substitution of tall battery jars to get rid of the excess humidity factor was only partly successful. Our experience led us to the opinion that the small germinator was of doubtful value for the purpose of this investigation.

Accordingly, our tests, for the most part, were carried on in the greenhouse, growing the plants in an ordinary greenhouse bench. The preliminary tests had indicated that a partial control for *Phoma betae* and other fungi could be expected with certain chemicals. Tests with soil were chosen because this would give opportunity to determine the value of the various treatments not only as seed disinfectants but as protective agents against organisms arising from a soil source as well.

The bed used was 34 inches wide and 6 inches deep. The rows were 3 inches apart, and in the first test 50 seed balls were planted 1 inch deep in each row. In all the subsequent tests, 30 seed balls were planted in a row at uniform depth by means of a simple planting device. A narrow strip of wood containing 30 holes 1 inch apart was placed across the leveled bed, a seed ball was placed in each hole and this was forced one inch below the surface by means of a plunger.

The sugar beet seed used in these greenhouse experiments was all from the same bag, and was American-grown seed obtained through the courtesy of the Holland-St. Louis Sugar Co. Samples of average sized seed balls were counted out, preliminary to any treatment, in an attempt to insure as nearly uniform a number of germs per row as possible, and to prevent any unconscious selection of seed balls on the basis of size. Each test was done in duplicate, the bed being divided into two sections. Every third row was used as a check row. The diseased seedlings were removed after the counts were taken. Daily records were taken and these continued for from three weeks to a month. The diseased seedlings were cultured on cornmeal agar plates following 1-2-minute immersion in HgCl_2 , 1-1000, and a rinse in sterile water unless otherwise indicated. The fungi developing were determined by microscopic examination.

Several complicating factors must be taken into account in experiments with sugar beet seeds. It must be borne in mind that the so-called sugar beet seed, the beet ball, is a cluster of embryos enclosed in the ovary wall along with remnants of the flower parts. The beet ball may contain from one to five or even more germs and close inspection is necessary to determine the number. The sorting of seed balls to a definite number of germs is not a practicable measure; accordingly, when 50 seed balls are planted, there will be considerable range in the number of seedlings emerging.

Then, too, the pathogens attacking beets spread from plant to plant, and the development of disease in a seedling may lead rather promptly to heavy loss of neighboring plants. Another complicating factor is the variation in the rate of emergence of the different sprouts. The germination of sugar beets planted at a uniform depth and given uniform watering stretches over a two-weeks period. The daily record taken from experimental rows shows a variation from day to day because some seedlings are lost by disease, and new seedlings are constantly coming up during the first part of the experimental period. In the records given in this paper we have summarized the daily readings to obtain a total of seedlings emerging and have made a similar summing up of the number of diseased seedlings found. On the last day of the test each row was counted, and this count checked, to determine the final stand. This stand has been compared with an assumed stand, such as would have been obtained from seed balls having two viable germs per ball under conditions when fungous attack was not a factor. We arrived at this figure as a fair normal to form a basis for judgment by many tests with average sized seed balls in sterile sand and sterile soil, after disinfecting the seed with various mercury disinfectants. The mean of many of these seed treatments showed approximately 1,000 seedlings from 500 seed balls.

EXPERIMENT I

Following the laboratory tests in germinators, various chemical treatments were carried on in the greenhouse, using a bench containing muck soil. This muck soil has been used for about two years for various vegetable crops, especially celery. The various treatments employed and the plan of the experiment is given in table 1 and illustrated in figure 1. This table shows the results obtained after a 23-day period. The last two columns show a ranking of the best treatments based (*a*) upon freedom from seedling disease and (*b*) upon stand.

It will be noted that the average germination of the check rows in this experiment was 55.5 ± 2.32 . The average number of diseased seedlings was 32 ± 1.47 . It will be noted that in spite of the variation in germination arising from beet balls of different sizes, as has been mentioned, these checks have not shown a very great range. Similarly the amount of damping-off which has occurred has fallen rather closely within the mean. It was not thought desirable to apply biometric analyses to other figures in the tables, since the results for the most part are so very outstanding. It seems safe to assume that we may expect a proportionate variation between the rows in the various treatments as is shown by the checks. It must be borne in mind, however, that where damping-off fungi occur in a clump of seedlings, the pathogens are likely to advance from seedling to seedling until

TABLE 1.—Comparison of various sugar beet seed treatments on muck soil; 50 seed balls planted per row. Record taken over a 23-day period following germination

Treatment	Number of seedlings emerging				Number of diseased seedlings				Per cent diseased				Per cent of "ideal stand," ^h				Averages		Rank based upon	
	Series A		Series B		Series A		Series B		Series A		Series B		Series A		Series B		Per cent diseased of stand ^b	Per cent of stand ^b	Freedom from disease	Stand
Check	63			33				52					30				52	30		
Copper sulphate and lime, ^a 50-50; dust, in excess	112	112		29		21		26	18			81	83				22	82	4	3
Copper carbonate, ^b 50 per cent; dust, in excess	99		89	44		6		44	6			83	45				25	64	6	6
Check	74	41		39		28		52	68			33	35				60	24		
Seed-O-San ^c	72	107		33		68		45	63			39	39				54	39		
Mercury bichloride, 1-1000; one hour soaking	93	93		10		24		10	25			69	83				18	76	3	4
Check	59	60		38		42		64	70			18	21				67	20		
Formaldehyde, 1-240; 30 min. soaking	55	46		28		29		50	63			17	27				57	22		
Chlorophol, ^d 1-400; one hour soaking	121	113		19		17		15	15			96	102				15	99	2	1
Check	69	59		27		36		39	61			23	42				50	33		
Kalimate	75	84		43		45		57	53			39	42				55	41		
Pythal, ^e 0.25 per cent; one hour soaking	96	98		15		10		15	10			88	81				13	85	1	2
Check	51	36		28		16		54	44			20	23				49	22		
Nickel carbonate; dust, in excess	73	83		58		59		79	71			22	15				75	19		
Copper carbonate, ^f 18 per cent; dust, in excess	93	91		18		23		19	25			68	75				22	72	5	5
Check	47	53		34		30		72	56			23	13				64	18		
Average of checks	55.5 ± 2.32		32 ± 1.47																	

^a Copper sulphate-lime dust was a mixture consisting of equal parts of copper sulphate (dehydrated) and hydrated lime. This and other dusts used in these tests were applied "in excess," i.e., all that would be carried by the seed. Application was made by shaking vigorously in a closed container for a few minutes.

^b Copper carbonate, 50 per cent, was furnished by the Dow Chemical Co., Midland, Michigan. This was the ordinary C. P. compound containing approximately 50 per cent metallic copper and was not finely ground. It did not adhere well.

^c Seed-O-San is an organic mercury compound furnished by Mr. M. O. Reiche, Chicago, Ill., and was used as a 0.25 per cent solution, for one hour.

^d Chlorophol is an organic mercury compound furnished by the Chicago Process Co., Chicago, Ill.

^e Kalimate is a proprietary compound containing formaldehyde, furnished by the Chicago Process Co., Chicago, and was used as a 0.25 per cent solution for one hour.

^f Pythal is an organic mercury compound containing phenol sold by the Chicago Process Co., Chicago, and contained gypsum as its chief diluent.

The analysis furnished by the company indicates an approximate metallic copper content of 18 per cent. It is sold under the trade name "Copper-carb." This dust adheres well and treated beet seed were conspicuously gray-blue.

^h Based upon assumed normal germination of two seedlings per seed ball.

TABLE 2.—Summary of tests of various sugar beet seed treatments on muck soil; 30 seed balls planted per row. *Revised taken over a period of 19 days*

No.	Treatment	Number of seedlings emerging		Number of diseased seedlings ^d		Per cent diseased		Per cent of "ideal stand"		Averages		Rank based upon	
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	Freedom from disease	Stand
I	Check	28	27	13	8	46	29	25	31	37.5	28.0		
II	Small seed balls ^a	18	14	3	3	16	21	25	15	19.0	20.0	3	
III	Large seed balls ^a	54	38	22	15	40	39	50	33	39.5	41.5		5
IV	Check	27	19	12	10	44	52	25	15	48.0	20.0		
V	Furfural, ^b 3 per cent	35	38	14	10	40	36	28	40	38.0	34.0		
VI	Furfural, ^b 1 per cent	30	27	11	15	36	55	33	18	44.5	25.5		
VII	Check	30	21	15	13	50	61	25	13	55.5	17.0		
VIII	Copper sulphate and lime dust	41	39	9	15	21	38	53	37	29.5	45.0	5	3
IX	Copper carbonate, 50 per cent	44	41	14	18	31	43	46	37	37.0	41.5		4
X	Check	36	33	13	16	36	48	38	28	42.0	33.0		
XI	Copper carbonate, 18 per cent	53	54	18	7	33	13	60	71	23.0	65.5	4	2
XII	Semesan, ^c dust	57	53	4	4	7	7	80	73	7.0	76.5	1	1
XIII	Check	35	28	9	12	25	42	43	26	33.5	34.5		
XIV	Large seed balls plus hydrated lime	29	30	10	9	34	30	28	28	32.0	28.0		
XV	Small seed balls plus hydrated lime	15	17	3	2	20	11	25	20	15.5	22.5	2	
XVI	Check	34	22	14	20	41	90	33	0	65.5	16.5		
XVII	Check		24		21		87		3				
	Average of checks	28 ± 1.02		13.5 ± 0.70									

^a Commercial seed was passed over a series of fine mesh to remove dust and abortive seeds. It was then separated by means of a slightly coarser screen (4 mm. mesh) into two classes, large and small seed. The small seed consisted largely of single germ seed balls; the large seed consisted of seed balls of 3 to 5 germs with a few 2-germ seed balls.

^b Furfural was obtained through the courtesy of the Miner Laboratory, Chicago, Ill., and was used as a soaking treatment at the two concentrations indicated after preliminary tests in germinators.

^c Semesan was furnished by E. I. du Pont de Nemours and Co., Wilmington, Del., and was recommended for use as a solution, or as a dust. Its active ingredient is an organic mercury compound. The analysis as given by the company is hydroxymethylchlorophenol sulfate 30 per cent, inert material 70 per cent. The active ingredient is readily soluble in the slightly alkaline solution which is formed as a result of the wetting of the components of the mixture. It is characteristic of the Semesan solution to be cloudy, and to deposit a small amount of inert material in the bottom of the container. Semesan was used unless otherwise noted as a dust treatment "in excess." The dust has excellent adherent qualities, and dusted seed is conspicuously gray-white in appearance, and feels slightly greasy to the touch.

^d Each diseased seedling was treated with HgCl₂, 1–1000, for 1–2 minutes and rinsed in sterile water before planting on cornmeal agar. In determining the organism, part of the growth was examined under the microscope. No distinction was made between *Pythium* and its close relatives. The following organisms were isolated from diseased seedlings in the groups indicated by number: I, III, V, IX, and XVI, *Pythium*; II, XI, and XII, *Pythium* and bacteria; IV, *Pythium* and *Rosellinia*; V, *Pythium* and *Sordaria*; VII and XIII, *Pythium* and *Fusarium*; VIII, *Pythium* and *Alternaria*; X, *Pythium* and *Aspergillus*; XIV, *Aspergillus* and *Cephalothecium*; XV, bacteria.

a considerable number of adjacent plants are affected. This is the probable explanation of erratic figures found in some of the succeeding tables.

As is clearly brought out by the two columns of table 1 in which the best of the treatments are ranked, nearly all of them were better than the checks, with the exception of the formaldehyde and the Kalimat (which contains formaldehyde as its active ingredient). Chloróphol, Pythal, and mercury bichloride, 1-1000, gave the greatest reduction in disease along with a high total germination. It is very evident that in disinfecting value the mercury compounds must be given first rank. The copper dust treatments gave an increase in germination over the check and a decrease in the percentage of diseased seedlings. Copper carbonate, 50 per cent dust, gave a poorer stand than copper carbonate, 18 per cent. The first mentioned compound was not ground to the same fineness as the 18 per cent mixture. The

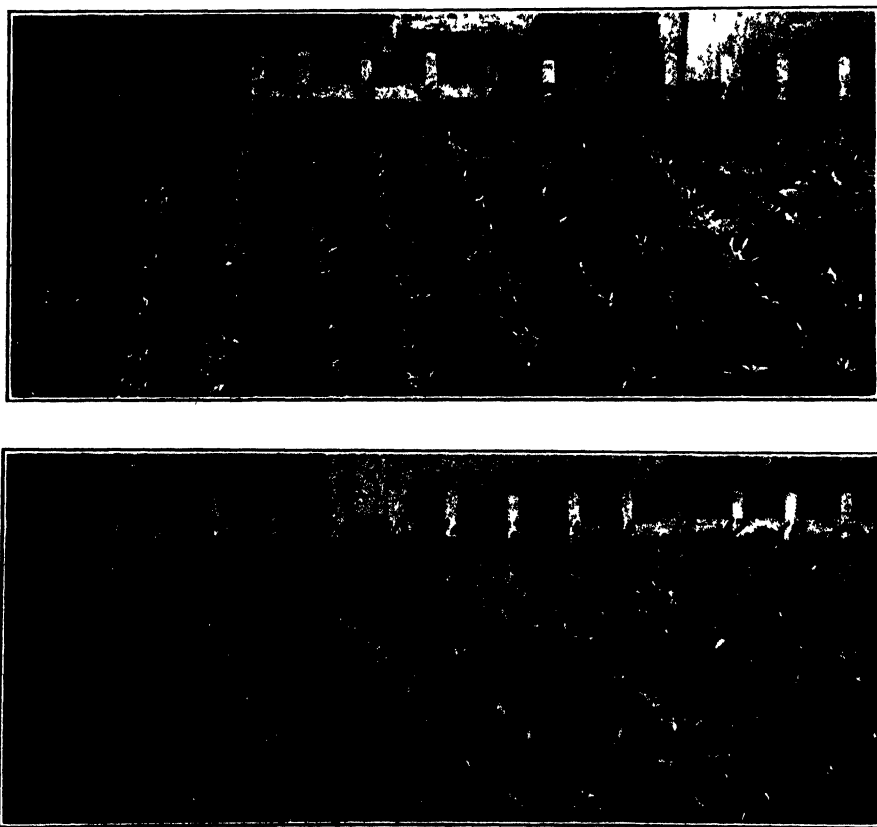


FIG. 1. Experiment 1. A comparison of various sugar beet seed treatments. Rows 1, 4, 7, 10, 13, and 16 were planted with untreated seed. The other rows were planted with seed treated as follows: 2, copper sulphate and lime, dust; 3, copper carbonate, 50 per cent, dust; 5, Seed-O-San; 6, mercury bichloride, 1-1000; 8, formaldehyde 1-240; 9, Chlorophol; 11, Kalimat; 12, Pythal; 14, nickel carbonate.

copper compounds in the form of a dust showed definite promise as a means of control of seedling disease (Fig. 2).

Formaldehyde, Kalimat, and Seed-o-san proved of no value in preventing seedling diseases after the plants have emerged from the soil. Nickel carbonate showed an injurious effect.

EXPERIMENT II

The method of planting was changed for Experiment II and 30 seed balls were used instead of 50. Beginning with this experiment each diseased seedling as removed was plated on cornmeal agar to permit determination of the organism responsible for the disease. The same soil which had previously been employed in Experiment I was used again. The general plan and the results of the test are given in table 2 and illustrated in figure 3.

In this test, the checks again show consistent behavior. Small and large seed balls, various copper compounds, and two concentrations of furfural were compared with the check.

It was thought possible that one of the effects of *Phoma* was the production of small seed balls because of a hypoplastic action upon the mother

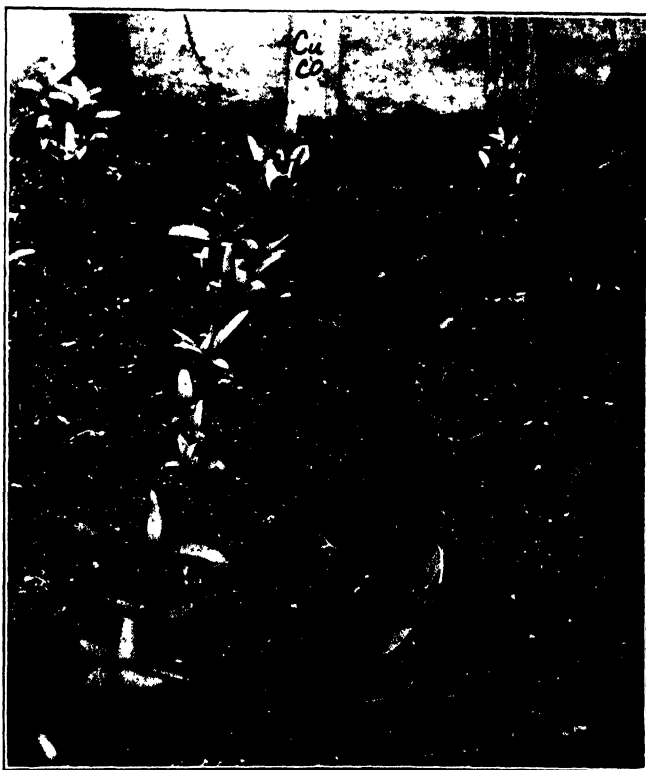


FIG. 2. Close view of rows 3 and 4 of Fig. 1, showing seedlings treated with copper carbonate dust contrasted with untreated seedlings. (Cf. table 1.)

plant. Experiment therefore was undertaken to determine the effect of discarding the moderately small seed balls. Preliminary experiments showed that *Phoma betae* occurred on the larger as well as smaller seed balls, and the results obtained in this experiment indicate that disease was still an important factor in reducing stand, and that the apparent gain by the use of large seed balls was simply due to the fact that there were a great many more germs in the case of the large seed balls than in the smaller ones.

The best treatment was the new mercury compound, Semesan, used as a dust in excess. It was best from a point of view of the reduction of disease and best also in stand. Copper compounds ranked about as before, but the faults of the coarse 50 per cent dust were magnified. Neither of the concentrations of furfural tried proved of any value and this compound was eliminated from future tests. Lime, which in some previous tests seemed to have slight promise, proved of no value when used with both the large and the small seed balls.

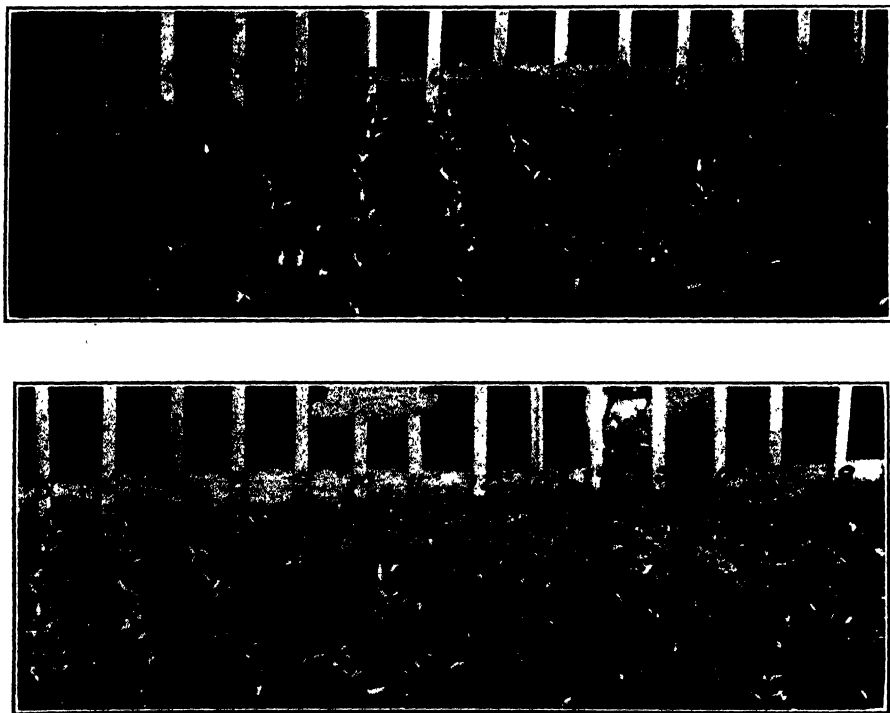


FIG. 3. Experiment II. Repetition of Experiment I with the same muck soil. Rows 1, 4, 7, 10, 13, and 16 were planted with untreated seed. The other rows were planted with seed treated as follows: 2, small seed balls dusted with lime; 3, large seed balls dusted with lime; 5, Semesan, dust; 6, copper carbonate, 18 per cent, dust; 8, copper carbonate, 50 per cent, dust; 9, copper sulphate and lime, dust; 11, furfural, 1 per cent; 12, furfural, 3 per cent. (Cf. table 2.)

The cultures made from the diseased seedlings gave very many *Pythium* spp. outgrowths. The other fungi and the bacterial growths did not appear consistently and were probably secondary. Not a single culture of *Phoma* or *Rhizoctonia* developed. It is possible that the preliminary disinfection with mercury bichloride influenced the flora which developed.

EXPERIMENT III

The experiments previously reported had been carried on with muck soil. To determine if the type of soil was responsible for the common occurrence of *Pythium*, as well as to take into consideration the various types of soil, sandy loam was substituted for the muck soil. This soil was obtained from a field on the College Farm where beans had been grown the previous summer. There is no record of sugar beets ever having been grown upon this soil. In this experiment various new mercury compounds were used in comparison with some of the chemicals found to be the more or less promising in the previous tests. The plan of the experiment is shown in table 3 and illustrated in figure 4.



FIG. 4. Experiment III. Sugar beet seed treated in various ways and planted in sandy loam soil. Rows 1, 2, 5, 8, 11, 14, and 17 were planted with untreated seed. The other rows were planted with seed treated as follows: 3, large seed balls, untreated; 4, Pythal; 6, copper sulphate and lime, dust; 7, mercury bichloride, 1-1000; 9, copper carbonate, 18 per cent, dust; 10, formaldehyde, 1-240; 12, Uspulun; 13, Semesan, dust; 15, Chlorophol; 16, Dupont Semesan 13, dust. (Cf. table 3, series B.)

TABLE 3.—*Summary of tests of various sugar beet seed treatments on sandy loam soil; 30 seed balls planted per row. Planting in duplicate. Record taken over a period of 13 days after germination began*

No.	Treatment	Number of seeds germinating				Number of seedlings diseased†				Per cent diseased				Per cent of "ideal stand"				Averages		Rank based upon	
		Series A		Series B		Series A		Series B		Series A		Series B		Series A		Series B		Per cent diseased		Per cent of stand	
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	Freedom from disease	Stand
I	Check	23	20	11	8	48	40	20	20	44	20	44	20	20	20	20	20				
II	Copper sulphate and lime, dust	19	26	0	8	0	31	31	30	15	30	15	30	30	30	30	30			4	
III	Mercury bichloride, 1-1000, 1 hr. soaking	39	53	6	4	15	7.5	55	81	11	68	11	68	81	81	81	81			2	5
IV	Check	16	12	4	4	25	33	20	13	29	16	29	16	20	13	13	16				
V	Copper carbonate, 18 per cent, dust	44	62	5	14	11	23	65	80	17	72	17	72	80	80	80	80			5	3
VI	Formaldehyde, 1-240; 1 hr. soaking	25	23	6	11	24	48	31	20	36	25	36	25	31	20	20	25				
VII	Check	35	13	10	5	28.5	38	41	13	33	27	33	27	41	13	13	27				
VIII	Uspulun, ^a 0.25 per cent; 2 hr. soaking	63	64	10	14	16	22	88	86	19	87	19	87	86	86	86	86			8	2
IX	Semesan, dust	72	62	15	9	21	14.5	95	88	18	91	18	91	95	88	88	91			6	1
X	Check	23	23	5	12	22	52	30	19	37	25	37	25	30	19	19	25				
XI	Chlorophol; 0.25 per cent; 1 hr. soaking	35	43	8	0	23	0	45	71	11	58	11	58	45	71	71	58			3	7
XII	Dupont Semesan 13 ^b , dust	41	49	7	2	17	4	57	79	10	68	10	68	57	79	79	68			1	4
XIII	Check	21	36	3	15	14	42	30	35	28	32	28	32	30	35	35	32				
XIV	Hulled seeds ^c	C	0																		
XV	Pasteurized 60° C.; 10 min. ^d	27	18	13	5	48	28	23	21	38	22	38	22	23	21	21	22				
XVI	Check	22	42	2	13	9	25	33	48	17	40	17	40	33	48	48	40				
XVII	Dry heat, 93° C.; 10 min.	18	26	7	15	39	57	18	18	48	18	48	18	18	18	18	18				
XVIII	Pasteurized 60° C.; 2 consecutive days; 10 min. each ^d	15	17	7	12	47	70	13	8	58	10	58	10	13	8	8	10				
XIX	Check	25	24	6	4	24	17	31	33	20	32	20	32	31	33	33	32				
XX	Dry heat, 110° C.; 10 min.	22	28	2	14	9	50	33	23	29	28	29	28	33	23	23	28				

TABLE 3.—Continued

No.	Treatment	Number of seeds germinating		Number of seedlings diseased		Per cent diseased		Per cent of "ideal stand"		Averages		Rank based upon
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	
XXI	Tillantin B, 0.25 per cent, 1 hr. soaking	13	31	8	17	61	55	8	23	58	15	8
XXII	Check	9	24	0	6	0	25	15	30	12.5	22	
XXIII	Tillantin C, 0.25 per cent; 1 hr. soaking	41	42	4	12	10	28	65	50	19	57	
XXIV	Small seed balls	6	9	3	6	50	67	5	5	58	5	6
XXV	Check	21	30	12	15	57	50	15	25	53	20	
XXVI	Large seed balls	21	54	9	33	42	61	20	40	51	30	
XXVII	Pythal, 0.25 per cent, 1 hr. soaking	39	51	3	16	8	31	60	61	19	60	7
XXVIII	Check	11	30	9	9	82	30	3	35	56	19	
XXIX	Check	30		13		43		29		43	29	
	Average of checks	23.3 ± 1.25		7.9 ± 0.64								

^a Uspulun was furnished through the courtesy of the Bayer Company, New York City, and is a mercury compound containing mercury-chlorophenol. It is recommended for use as a 0.25 per cent solution.

^b Dupont Semesan 13 is an organic mercury compound containing approximately 10 per cent of hydroxymercurichlorophenol, according to analysis furnished by the manufacturer.

^c Seed balls were crushed and apparently sound seeds selected in an attempt to secure viable material free from the contaminated plant parts.

^d Seed balls were placed in water at 60° C. in a water bath. After 10 minutes exposure the seeds were divided on blotting paper at 30° C.

^e Tillantin B and Tillantin C were furnished by H. A. Metz and Co., New York City. The former is a copper compound containing arsenic, and the latter an organic mercury compound.

^f Diseased seedlings were planted directly upon cornmeal-agar plates without preliminary disinfection, since it was believed that the mercury bichloride affected the flora developing. The following organisms were isolated from diseased seedlings in the groups indicated by Roman numerals (the number in parenthesis denotes the number of times the organism was isolated): I, IV, VII, X, XIII, XVI, XIX, XXII, XXV, XXVIII, XXIX, (the check rows), *Pythium* (7), *Ehizoctonia* (5), *Phoma* (1); II, none; III, *Pythium* (1); V, *Pythium* (1), *Phoma* (1), *Fusarium* (1); VI, *Mucor* (1), *Fusarium* (1), *Pythium* (1); VII, *Mucor* (1), *Pythium* (3); IX, *Mucor* (2); *Pythium* (1), *Ehizoctonia* (1), *Fusarium* (1); XI, *Pythium* (1); XII, *Pythium* (1), *Pythium* (2), *Mucor* (2), *Mucor* (1), *Pythium* (1); XIII, *Pythium* (1); XVII, *Pythium* (1); XVIII, *Mucor* (1), *Alternaria* (1); XX, *Pythium* (2); XXI, *Pythium* (3); XXIII, *Pythium* (1); XXIV, *Pythium* (1), *Mucor* (1); XXVI, *Mucor* (1), *Pythium* (2); XXVII, *Pythium* (1), *Mucor* (2), *Fusarium* (1).

The average total germination of the check rows was 23.3 ± 1.25 . The average total diseased seedlings in the checks was 7.9 ± 0.64 . These figures are very consistent considering the condition of the test.

It is evident that again the mercury compounds showed the highest total germination and the lowest percentage of disease. The DuPont Semesan 13 showed the fewest diseased seedlings. Chlorophol and mercury bichloride, used as soaking treatments, had approximately the same number of diseased seedlings. On the basis of final stand calculated as percentage of assumed normal stand, Semesan and Uspulun—the former as a dust and the latter as a soaking treatment—rank first and second, respectively. Copper carbonate ranks third, with other compounds following rather closely. The copper sulphate lime dust was not strikingly better than the check in final stand. Tillantin B., a copper compound containing arsenic, was of no value. Soaking the seed balls in formaldehyde 1-240 was of no value.

Pasteurization at 60° C. for ten minutes on one day, and pasteurization on two successive days gave no indication of controlling seedling diseases. While it is possible that the treatment reduced the *Phoma betae* infestation to a great extent, the seedlings were not protected against soil organisms. Dry heat at 93° C. or 110° C. for ten minutes proved of no value as a means of preventing disease.

The separation of the seed balls into two types, small and large, proved to be of no value, confirming the previous test. Attempts to hull the seed were a complete failure. None of the seeds germinated, probably due to injury done in hulling.

In general it may be said that every chemical controlling disease and giving a stand better than the check stand was either a mercury or copper compound.

All seedlings which became diseased in the last six days of the test were plated. Considering that the duplicate rows were one-half the distance of the bed from its corresponding treatment, it will be seen from table 3 that the fungi affecting the seedlings were uniformly distributed throughout the soil; especially was this true of *Pythium*. Seven cultures of *Rhizoctonia* were obtained, five of these occurring in the untreated rows. Three cultures of *Phoma* were found, one from an untreated row. *Mucors*, *Fusaria*, and *Alternaria* were found, but these are looked upon as saprophytes playing but a slight rôle in the seedling disease problem.

EXPERIMENT IV

In order to repeat Experiment III and to demonstrate in miniature the effect of continuous cropping of beets, the soil was used again for planting. The seedlings were removed and the soil thoroughly mixed. It was then

planted to beets. The plan of the experiment is shown in table 4 and illustrated in figure 5. The results are summarized from 17 days' record.

A comparison of the check rows of Experiments III and IV is interesting. The most striking thing about the results is the marked reduction in stand and the increase of disease in the check plats of Experiment IV as compared with the previous experiment. Conditions within the greenhouse, and water relations of the bed were not noticeably different in the two experiments, and we feel secure in attributing the differences in stand to the intensification of pathogens which came from the preceding crop.

The 21 untreated rows in Experiment IV showed a total germination of 8 ± 0.51 seedlings as contrasted with 23.3 ± 1.25 in Experiment III.

Assuming an average of 2 germs per seed ball, these figures show that the total number of seedlings emerging was only 13 per cent of the normal as contrasted with 38 per cent in the former test. In Experiment IV, 61

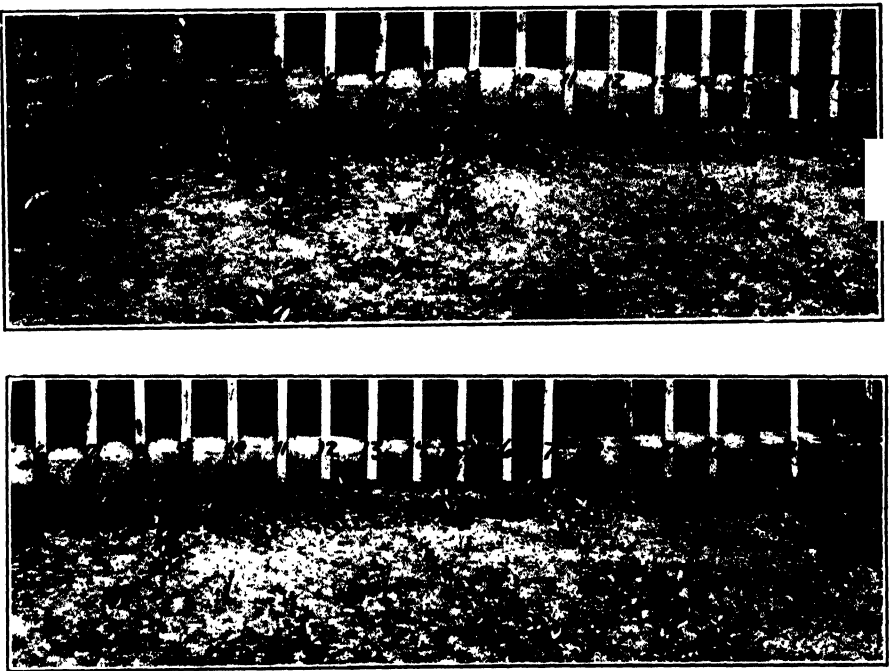


FIG. 5. Experiment IV, a. Repetition of seed treatment Experiment III using the sandy loam soil a second time for seedlings. Rows 3, 6, 9, 12, 15, 18, 21, and 24 were planted with untreated seed. The other rows were planted with seed treated as follows: 1, Tillantin C; 2, small seed balls, untreated; 4, large seed balls, untreated; 5, Pythal; 7, copper sulphate and lime, dust; 8, mercury bichloride, 1-1000; 10, copper carbonate, 18 per cent, dust; 11, formaldehyde, 1-240; 13, Uspulun; 14, Semesan, dust; 16, Chlorophol; 17, Dupont Semesan 13, dust; 19, hulled seed; 20, pasteurized seed balls; 22, dry heat, 93° C. (Cf. table 4, series B.)

TABLE 4.—*Repetition of sugar beet seed treatments outlined in table 3; same sandy loam soil used after thorough mixing. Record taken over a period of 17 days after germination began*

No.	Treatment	Number of seeds germi- nating		Number of seedlings diseased*		Per cent diseased		Per cent of "ideal stand,"		Averages		Rank based upon	
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	Freedom from disease	Stand
I	Check	5	10	1	7	20	70	6	11	45	8		
II	Copper sulphate and lime, 50-50, dust, in excess	16	54	1	33	6	61	25	35	33	30	8	8
III	Mercury bichloride, 1-1000, 1 hr. soaking	43	37	14	11	32	30	48	43	31	45	5	4
IV	Check	5	12	2	6	40	50	5	10	45	7		
V	Copper carbonate, (18 per cent) dust in excess	38	31	9	13	23	41	48	30	32	39	7	6
VI	Formaldehyde, 1-240; 1 hr. soaking	9	2	3	1	33	50	10	1	41	5		
VII	Check	5	12	1	7	20	58	6	8	39	7		
VIII	Uspulun 0.25 per cent; 2 hr. soaking	32	39	11	7	34	18	35	53	31	44	4	5
IX	Semesan; dust in excess	49	47	12	11	24	23	61	60	23	60	3	2
X	Check	5	8	1	5	20	62	6	5	41	5		
XI	Chlorophol, 10 gm. per gal., 1 hr. soaking	35	50	2	6	5	12	55	73	8	64	1	1
XII	Dupont Semesan, No. 13; dust in excess	48	53	13	20	27	38	58	55	32	56	6	3
XIII	Check	8	3	2	3	25	100	10	0	62	5		
XIV	Hulled seeds	0	0										
XV	Pasteurized 60° C.; 10 min.	5	8	5	7	100	87	0	0	93	0		
XVI	Check	12	6	3	3	25	50	5	1	37	3		
XVII	Dry heat, 93° C.; 10 min.	18	12	14	11	78	92	6	1	85	3		
XVIII	Pasteurized 60° C.; 10 min. on 2 consecutive days	9	5	8	4	89	80	1	1	84	1		

TABLE 4.—Continued

No.	Treatment	Number of seeds germi- nating		Number of seedlings diseased ^a		Per cent diseased		Per cent. of "ideal stand"		Averages		Rank based upon	
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	Freedom from disease	Stand
XIX	Check	10	5	6	5	60	100	6	0	80	3		
XX	Dry heat, 110° C.; 10 min.	4	7	3	4	75	57	1	5	66	3		
XXI	Tilantin B, 0.25 per cent, 1 hr. soaking	22	10	12	7	55	70	16	5	62	10		
XXII	Check	8	9	3	5	38	56	8	6	47	7		
XXIII	Tilantin C, 0.25 per cent, 1 hr. soaking	20	36	13	10	65	28	11	43	46	27		
XXIV	Small seed balls	3	3	0	1	0	33	5	3	16	4		
XXV	Check	6	13	3	3	50	23	5	5	36	5		
XXVI	Large seed balls	22	18	10	12	45	67	20	10	56	15		
XXVII	Pythol, 1 per cent, 1 hr. soaking	36	21	3	5	8	23	55	26	15	35	2	7
XXVIII	Check	4	10	4	9	100	90	0	1	95	0		
XXIX	Check		9		8		89		1	89	1		
	Average of checks	8 ± 0.51		4.9 ± 0.43									

^a The following organisms were isolated from diseased seedlings in the groups indicated by Roman numeral (the number in parenthesis denotes the number of times the organism was isolated): I, IV, VII, X, XIII, XVI, XIX, XXII, XXV, XXVIII, XXIX (the check rows), *Pythium* (25), *Rhizoctonia* (8), *Phoma* (1), *Mucor* (18), *Fusarium* (18), miscellaneous (7). II, Series A, *Pythium* (1); Series B, *Pythium* (6), *Mucor* (6), *Phoma* (1), *Fusarium* (1), *Rhizoctonia* (11). III, Series A, *Pythium* (2), *Fusarium* (4), *Mucor* (3), *Penicillium* (1); Series B, *Phoma* (1), *Fusarium* (3), *Pythium* (3), *Mucor* (3). V, Series A, *Fusarium* (6), *Pythium* (3); Series B, *Fusarium* (3), *Mucor* (3), *Pythium* (7). VI, Series A, *Pythium* (1), *Mucor* (1), *Fusarium* (1); Series B, none. VIII, Series A, *Pythium* (5), *Mucor* (1), *Fusarium* (3); Series B, *Rhizoctonia* (1), *Mucor* (2). IX, Series A, *Pythium* (3), *Fusarium* (4); Series B, *Phoma* (4), *Fusarium* (4), *Pythium* (1). XI, Series A, *Fusarium* (1), *Mucor* (1); Series B, *Pythium* (3), *Rhizoctonia* (1), *Sordaria* (1). XII, Series A, *Pythium* (8), *Mucor* (3), *Fusarium* (1); Series B, *Pythium* (6), *Mucor* (1). XV, Series A, *Rhizoctonia* (1), *Fusarium* (3); Series B, *Pythium* (2), *Fusarium* (4), *Phoma* (1), *Mucor* (2). XVII, Series A, *Phoma* (1), *Fusarium* (4), *Mucor* (3), *Pythium* (4)⁴; Series B, *Pythium* (2), *Fusarium* (4), *Phoma* (1), *Mucor* (2). XVIII, Series A, *Pythium* (3), *Mucor* (1), *Fusarium* (2); Series B, *Fusarium* (3), *Pythium* (1). XX, Series A, *Pythium* (1), *Alternaria* (1); Series B, *Pythium* (2), *Mucor* (1). XXI, Series A, *Pythium* (4), *Mucor* (4), *Fusarium* (2); Series B, *Pythium* (1), *Alternaria* (1), *Fusarium* (1). XXIII, Series A, *Fusarium* (6), *Mucor* (2), *Pythium* (2); Series B, *Fusarium* (6), *Pythium* (2), *Mucor* (3). XXIV, Series B, *Mucor* (1). XXVI, Series A, *Pythium* (4), *Fusarium* (4), *Mucor* (2); Series B, *Pythium* (2), *Fusarium* (4), *Rhizoctonia* (1), *Mucor* (1). XXVII, Series A, *Pythium* (2); Series B, *Pythium* (1),

per cent of the seedlings emerging became diseased, and the final stand on the average was only 5 per cent of the assumed normal stand. In Experiment III approximately 33 per cent of the seedlings which emerged became diseased and the final stand was 25 per cent of the assumed normal stand.

These figures illustrate in forcible manner the influence a diseased crop may have upon a succeeding crop. With such soil infestation, it is obvious that the various seed treatments are put to extreme tests as to their capacity for protecting the young seedling as it emerges and grows into the soil.

With every treatment the stand in Experiment IV is poorer than in Experiment III and the percentage of disease is higher. The results with the various seed treatments are gratifying, however, considering the soil infestation. The superior treatments of Experiment III stand high also in Experiment IV. Based upon percentage of assumed normal stand, the treatments have the following position: Chlorophol, Semesan dust, Du Pont Semesan 13, mercury bichloride (1-1000), and Uspulun (0-25 per cent). The copper compounds and Pythol gave from 30 to 40 per cent of the possible startfl with a disease percentage ranging from 15 in the case of Pythal to 33 in the case of copper sulphate-lime dust. The other treatments gave no indications of control, thus confirming previous tests.

A large number of *Pythium* cultures were obtained from the diseased seedlings. In fact, *Pythium* appeared as frequently as with the muck soil used in Experiment II. In a few instances *Phoma* and *Rhizoctonia* were found; *Rhizoctonia* was more common on the untreated rows. A large number of growths of *Fusarium* and *Mucor* developed.

EXPERIMENT V

In order to compare the influence of the seed-borne organisms with those arising from the soil in their effects upon stand and upon the prevalence of disease, an experiment was planned in which the sandy loam soil previously used was left in one half of the greenhouse bed and the other half of the bed, after disinfection with strong formaldehyde, was filled with clean sand. The most satisfactory treatments of the preceding tests were chosen, and pasteurization at 60° C. for 10 minutes on two successive days was also included because of the reports that have been given as to its value in eliminating *Phoma betae*. The plan of the experiment with sandy loam soil along with the summary of the record at the close of a 14-day period is given in table 5 and illustrated in figure 6. Table 6 and figure 7 give similar data for the portion of the bed containing clean sand.

The results of this experiment can probably best be made clear by contrasting the behavior of the untreated seed in soil and sand. The total

germination of the checks in sand was 35.0 ± 0.81 and of these 11.4 ± 1.00 were diseased. On the other hand, the untreated seed in the soil series showed a total germination of only 10.1 ± 0.67 seedlings of which 5.1 ± 0.40 were diseased. These figures show in a striking way the rôle that soil organisms play in the production of seedling diseases.



FIG. 6. Experiment V. Sandy loam soil series of seed treatments. Rows 1, 2, 5, 8, 11, 14, and 17 were planted with untreated seed. The other rows were planted with seed treated as follows: 3, copper sulphate and lime, dust; 4, mercury bichloride, 1-1000; 6, copper carbonate, 18 per cent, dust; 7, pasteurized; 9, Chlorophol; 10, Semesan, dust; 12, Uspulun; 13, Dupont Semesan 13; 15, copper sulphate and lime, dust. (Cf. table 5, series A.)



FIG. 7. Experiment V. Clean sand series of seed treatments. The rows in this test duplicate those given in the legend of figure 6. (Cf. table 6, series A.)

TABLE 5.—Summary of sugar beet seed treatments on sandy loam soil: third planting on same soil. Period, 14 days after germination

No.	Treatment	Number of seeds germinating				Number of seedlings diseased ^a				Per cent of "ideal" stand				Averages		Rank based upon	
		Series A		Series B		Series A		Series B		Series A		Series B		Per cent diseased	Per cent of stand	Freedom from disease	Stand
I	Check	14	11	6	8	43	73	13	5	58	9						
II	Copper sulphate-lime; dust in excess	18	22	6	10	33	45	20	20	39	20					6	7
III	Mercury bichloride, 1-1,000; 1 hr. soaking	15	22	10	6	66	23	25	26	44	25					7	6
IV	Check	15	9	6	3	60	33	15	10	46	12						
V	Copper carbonate, 18 per cent; dust in excess	19	34	4	13	21	38	25	35	29	30					4	5
VI	Pasteurized; 60° C., 20 min.	9	13	5	5	55	38	6	13	46	9						
VII	Check	12	15	7	6	58	60	8	15	59	12						
VIII	Chlorophol, 0.25 per cent; 1 hr. soaking	40	49	7	4	17	8	55	75	12	65					1	2
IX	Semesan; dust in excess	50	50	12	15	24	30	63	58	27	60					3	3
X	Check	8	8	2	6	25	75	10	3	50	6						
XI	Uspulun, 0.25 per cent; 2 hrs. soaking	44	38	12	17	27	45	53	35	36	44					5	4
XII	Dupont Semesan, No. 13; dust in excess	47	46	4	9	9	19	71	61	14	66					2	1
XIII	Check	8	5	4	3	50	60	6	3	55	4						
XIV	Check		7		6	85				85							
	Average of checks	10.1 ± .67		5.1 ± .4													

^a The following organisms were isolated from diseased seedlings in the groups indicated by Roman numeral (the number in parenthesis denotes the number of times the organism was isolated): I, IV, VII, X, XIII, XIV (the check rows), *Pythium* (29), *Fusarium* (9), *Bacteria* (2). II, Series A, *Pythium* (5), *Fusarium* (1); Series B, *Pythium* (3), *Phoma* (1), *Fusarium* (1). III, Series A, *Fusarium* (2), *Pythium* (6); Series B, *Pythium* (2), *Fusarium* (2). V, Series A, *Fusarium* (1), *Pythium* (2); Series B, *Pythium* (11). VI, Series A, *Phoma* (1), *Pythium* (4); Series B, *Pythium* (2), *Fusarium* (3). VIII, Series A, *Pythium* (2), *Bacteria* (2); Series B, *Pythium* (2). IX, Series A, *Pythium* (5), *Fusarium* (2); Series B, *Pythium* (9), *Fusarium* (2). XI, Series A, *Pythium* (8); Series B, *Pythium* (4), *Rhizoctonia* (2), *Fusarium* (3). XII, Series A, *Pythium* (2), *Fusarium* (2); Series B, *Pythium* (6), *Fusarium* (3).

TABLE 6.—Summary of sugar beet seed treatments on clean sand: for comparison with tests given in table 5. Period, 14 days after germination

No.	Treatment	Number of seeds germinating		Number of seedlings diseased ^a		Per cent diseased		Per cent of "ideal" stand		Averages		Rank based upon
		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B	Per cent diseased	Per cent of stand	Freedom from disease
I	Check	35	31	11	21	31	68	40	16	49	28	
II	Copper sulphate-time; dust in excess	39	40	12	10	31	25	45	50	28	57	8
III	Mercury bichloride, 1-1,000; 1 hr. soaking	39	62	7	6	18	10	55	91	14	73	5
IV	Check	31	37	14	5	45	13	28	53	29	40	
V	Copper carbonate, 18 per cent; dust in excess	43	47	6	8	14	18	61	65	16	63	7
VI	Pasteurized; 60° C., 20 min.	53	55	3	1	5	2	83	90	3	86	2
VII	Check	37	33	13	12	35	36	40	35	35	37	
VIII	Chlorophol, 0.25 per cent; 1 hr. soaking	39	43	0	1	0	2	65	70	1	67	6
IX	Semesan; dust in excess	58	82	1	4	2	5	95	130	3	112	1
X	Check	43	28	9	12	21	42	56	26	31	41	
XI	Uspulun, 0.25 per cent; 2 hrs. soaking	51	55	2	6	4	11	81	81	7	81	3
XII	Dupont Semesan, No. 13; dust in excess	51	47	0	3	0	6	85	73	3	79	4
XIII	Check	38	38	3	10	8	26	58	46	17	52	
XIV	Check		36		16		44		33	44	33	
Average of checks		35.0 ± .81		11.4 ± 1.00								

^a The following organisms were isolated from diseased seedlings in the groups indicated by Roman numeral (the number in parenthesis denotes the number of times the organism was isolated: I, IV, VII, X, XIII, XIV (the check rows), *Phoma* (106), *Pythium* (3), *Alternaria* (2), *Bacteria* (4). II, Series A, *Phoma* (12); Series B, *Phoma* (10). III, Series A, *Phoma* (5); Series B, *Phoma* (6). V, Series A, *Phoma* (3); Series B, *Phoma* (6). VI, Series A, *Phoma* (3); Series B, *Phoma* (1). VII, Series B, *Bacteria* (1). VIII, Series A, *Phoma* (1); Series B, *Phoma* (2). IX, Series A, *Phoma* (1); Series B, *Phoma* (2). XI, Series A, *Phoma* (1); Series B, *Phoma* (4). XII, Series B, *Phoma* (2).

In the soil series in spite of the heavy infestation with pathogens, the mercury compounds still showed considerable control and the figures obtained are approximately the same as those obtained in Experiment IV. The copper sulphate-lime dust was not so satisfactory as in former tests. Copper carbonate was superior to the soaking treatment with mercury bichloride. The pasteurized seed was but slightly better than the untreated seed.

When the data from the soil and sand experiments are compared, some outstanding things are shown. First, seedlings receiving the pasteurization treatment which had proved to be of no value in the soil tests now ranked second in stand and fourth in freedom from disease in the sand planting. Semesan was the outstanding chemical in this test, giving a stand better than the assumed normal. In disease reduction Chlorophol ranks first, with Du Pont Semesan 13, Semesan, and pasteurization treatments showing approximately the same number of diseased seedlings and the same percentage diseased of the total number germinating. Copper carbonate again compares favorably with mercury bichloride soaking treatment.

The results from the untreated rows on sand give some idea as to the real importance of *Phoma betae*. Thirty-three per cent of the seedlings appearing above the surface of the sand became diseased and with few exceptions *Phoma* was responsible. Three cultures of *Pythium* were found but they came from the buffer row nearest the row of bricks which separated the sand from the soil portion of the bed. Based upon the assumed normal number that should have appeared if *Phoma* had been eliminated, 44 per cent of the seedlings were killed below the surface of the ground and 11.4 of those emerging contracted *Phoma* disease. It is doubtless true that *Phoma* was actively concerned in the death of seedlings in the previous experiments but the rapidity of growth of other organisms and the mixed infections masked the situation.

The writers assume that the *Phoma* in this experiment came from the seed ball. No examination of the sand used in this experiment was made, but it seems that it may be safely eliminated as a source of the fungus, since the sand was fresh from the sand pit and had no chance of being contaminated with beet debris.

This test shows that beet balls can not be completely freed from *Phoma betae* by any one of the treatments tried with the possible exception of Chlorophol. This chemical has not given a very good stand. The results with the mercury compounds, however, are promising because of the marked disease reduction. Copper carbonate also has given fair results.

EXPERIMENT VI

In the experiment just reported, clean sand and infested sandy loam soil were compared in their general effect on stand and percentage of dis-

ease. In order to determine if the physical nature of the substratum played any rôle in the results obtained in the previous planting the following experiment was performed.

The soil and the sand used in Experiment V was autoclaved at 15 pounds pressure for an hour. New greenhouse flats, 16 by 20 by 3 inches, were filled with the sterilized soil and the sterilized sand. Similar flats were prepared with the non-sterile soil and non-sterile sand. Three rows of 30 seed balls each were planted in each of the four flats. The middle row in each case was planted with seed balls which had been dusted with Semesan as a dust (in excess). The record taken 12 days after germination began showed the results given in table 7.

The results of this test are entirely confirmatory of the previous tests and indicate that the important factor in the production of the disease of the seedlings is not the physical character of the soil but rather its fungous content. The results with sterilized soil and sterilized sand are very similar and the non-sterile sand showed approximately the readings of the autoclaved sand. In non-sterile soil the stand was almost a complete failure in the case of the untreated seed, and only 16 seedlings grew in the case of the

TABLE 7.—*Comparison of sugar beet seedling diseases on autoclaved soil and sand and on non-sterile soil and sand: Tests made in flats, 3 rows per flat, 30 seed balls per row. Period 18 days*

	Seed treatment	No. of seedlings emerged	No. of seedlings diseased
Autoclaved sand	Untreated	25	4
	Semesan*	56	2
	Untreated	28	2
Autoclaved soil	Untreated	28	5
	Semesan*	58	2
	Untreated	33	3
Non-sterile sand	Untreated	27	3
	Semesan*	66	1
	Untreated	40	2
Non-sterile soil	Untreated	2	0
	Semesan*	16	1
	Untreated	1	0

* Semesan used as a dust in excess.

Semesan treatment. This was the fourth crop of seedlings for this soil, and a comparison of the results with those of the preceding experiments shows the progressive increase in disease organism contamination. Here again the

Semesan treatment was put to an extremely severe test: the stand resulting was only 27 per cent of the assumed normal, a gain, however, of approximately 24 per cent over the checks. A study of the results with treated and untreated seed obtained with sand, both sterile and unsterile, and with the autoclaved soil shows that the organisms carried on the seed play an exceedingly important rôle in influencing stand, the germination of the treated seed being approximately double that of the untreated seed.

EXPERIMENT VII

To compare the pathogenicity of the three principal organisms concerned with damping-off of the beet seedlings, inoculation experiments were performed with organisms from the following sources:

1. *Phoma betae* isolated from a diseased seedling growing in sterile sand.
2. *Phoma betae* from diseased sugar beet seed from Colorado.
3. *Rhizoctonia* spp. from a diseased seedling.
4. *Rhizoctonia* spp. from laboratory stock culture isolated from potato.
5. *Pythium debaryanum* isolated from a diseased seedling growing in soil.

About three weeks before the seed balls were planted the organisms named above were planted on sterilized cornmeal in liter flasks. Clean building sand was sterilized, and the greenhouse flats were disinfected by soaking in formaldehyde 1-10. The cornmeal, which in three weeks became covered and impregnated with the fungous growth, was thoroughly mixed with the sterile sand. Two rows of untreated seed and one row of seed dusted with Semesan were planted in each flat, 30 seed balls per row. The results of the experiment are given in table 8 and illustrated in figure 8.

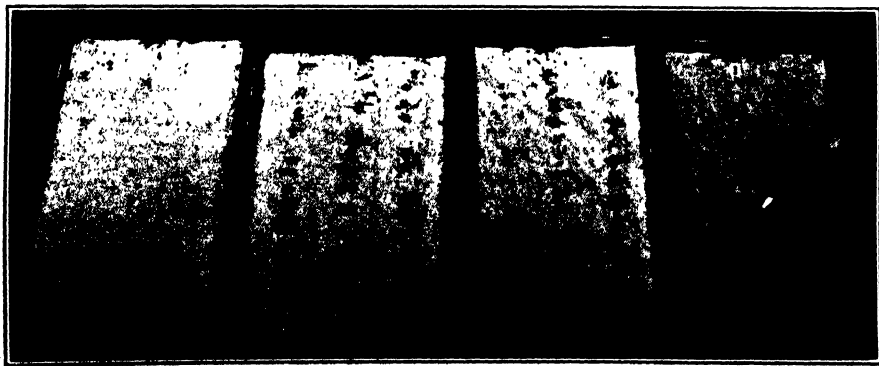


FIG. 8. Flats of sterilized sand to which cultures of various organisms were added three weeks before the seed balls were planted compared with a flat of sterile sand. Photographed three weeks after the seed was planted.

Flat 1: Check. 2: *Rhizoctonia* from a beet seedling. 3: *Pythium* spp. from a beet seedling. 4: *Phoma betae*.

TABLE 8.—*Inoculation experiments with three sugar beet disease organisms. Sterile sand in flats inoculated by adding 3-weeks-old cultures of the organism on cornmeal*

Flat no.	Organism used to inoculate flats	Treatment given seed balls	No. of seedlings emerging	No. of seedlings diseased	Organisms isolated from diseased seedlings
1	<i>Phoma betae</i> obtained from beet seedling	Untreated Semesan ^a Untreated	1 1 0	1 1 —	<i>Phoma</i> (1) <i>Phoma</i> (1)
2	<i>Phoma betae</i> isolated from a sugar-beet grown in Colorado	Untreated Semesan Untreated	0 7 2	— 7 2	<i>Phoma</i> (6) <i>Mucor</i> (1)
3	<i>Pythium debaryanum</i> obtained from a seedling	Untreated Semesan Untreated	0 0 0	— — —	
4	<i>Corticium vagum solani</i> (''Rhizoctonia'')	Untreated Semesan Untreated	16 52 39	2 15 9	<i>Rhizoctonia</i> (2) <i>Rhizoctonia</i> (13), no growth (1), <i>Alternaria</i> (1) <i>Rhizoctonia</i> (9)
5	<i>Corticium vagum solani</i> from potato. (''Rhizoctonia'')	Untreated Semesan Untreated	25 39 34	7 11 12	<i>Rhizoctonia</i> (4), <i>Mucor</i> (2) <i>Rhizoctonia</i> (5), <i>Mucor</i> (2) <i>Rhizoctonia</i> (4), <i>Mucor</i> (2)
6	Mixture of cornmeal cultures of <i>Phoma betae</i> , <i>Pythium debaryanum</i> , <i>Corticium vagum solani</i> used in flats 1, 3, and 4; light inoculation	Untreated Semesan Untreated	9 26 11	5 20 8	<i>Rhizoctonia</i> (5), <i>Pythium</i> (1) <i>Phoma</i> (2), <i>Pythium</i> (2), <i>Rhizoctonia</i> (12) <i>Phoma</i> (1), <i>Rhizoctonia</i> (6)
7	Sterile cornmeal—check	Untreated Semesan Untreated	30 34 26	6 3 10	<i>Mucor</i> (4), <i>Alternaria</i> (1) <i>Bacteria</i> (1), <i>Mucor</i> (1) <i>Phoma</i> (2), <i>Mucor</i> (5), <i>Bacteria</i> (1)
8	Sterile sand—check	Untreated Semesan Untreated	40 44 44	5 0 1	<i>Phoma</i> (1), <i>Bacteria</i> (3), <i>Alternaria</i> (1) <i>Mucor</i> (1)

^a Semesan dust was used in excess.

In the flat inoculated with *Pythium* no seedlings appeared. Even the row of seed balls treated with Semesan failed completely. Examination showed that the seed had sprouted but the young seedlings were killed by *Pythium* before reaching the surface of the soil. With such heavy inoculation the Semesan was not able to ward off the organism. It is possible that with the sand as a substratum the toxic ingredients of the Semesan were more widely dissipated than they would have been if the finer and more absorptive soil particles were present to bind the heavy metal by adsorption.

Phoma betae was almost as severe in its effects as *Pythium*. A few seedlings came above the surface in each of the two tests with *Phoma*, but these soon became diseased. Under the conditions of this test, Semesan failed to protect the young plants.

The flats inoculated with *Rhizoctonia* gave results at marked variance with those inoculated with *Pythium* and *Phoma*. The inoculum which was applied to the soil soon gave rise to a very heavy growth of the organism. This was very evident even before the seedlings emerged from the sand, for the fungus had grown so strongly as to crust the soil. It will be noted in table 8 that a considerable number of seedlings appeared. The results after the first 12 days seemed to indicate that *Rhizoctonia* was a rather weak or at least a slowly working parasite. Twenty-four days after the germination of the seedlings began, the flats were discarded. Special attention was given to the *Rhizoctonia*-inoculated flats and each seedling was carefully washed out of the sand and examined. It was found that almost every seedling from the check rows in both flats had a decayed root tip (Figure 9). The Semesan rows were slightly better than the check rows. The strain of *Rhizoctonia* from potato was as effective as the strain isolated from the beet seedling. Where the root tip was decayed the plant had thrown out from 3 to 8 small rootlets just above the decayed region to compensate for the loss of the primary root system. The hypocotyl was sound above the surface of the sand but the leaves were not of normal color. A few showed signs of wilting. It seemed extremely likely that in many cases the seedling would have succeeded in replacing the diseased portion and would have remained alive. The resulting beet root would have been forked and sprangled, and it seems extremely probable that the forked and sprangled roots so common in some fields at harvest time which have been attributed to high water table or impervious subsoil are more properly to be attributed to *Rhizoctonia* which has been influenced by the above named factors.

The results with the mixture of all of the organisms gave some results of considerable interest. The inoculum for this flat was made by taking a small amount of the sand from flats 1, 3, and 4 and mixing thoroughly.

The amount of inoculum added was small. A greater number of seedlings emerged in the untreated rows in this flat and Semesan had slightly more influence in warding off disease. In the isolations only a few cultures of *Phoma betae* and *Pythium* spp. were obtained, *Rhizoctonia* spp. being by far the most common. This at first glance seems contradictory considering the strong pathogenicity of *Pythium* spp. and *Phoma betae* shown in the other flats. The plausible explanation is that the seedlings attacked by *Pythium* spp. and *Phoma betae* were killed before reaching the surface. The seedlings which survived probably escaped *Pythium* and *Phoma betae* but did harbor the slower working *Rhizoctonia*.

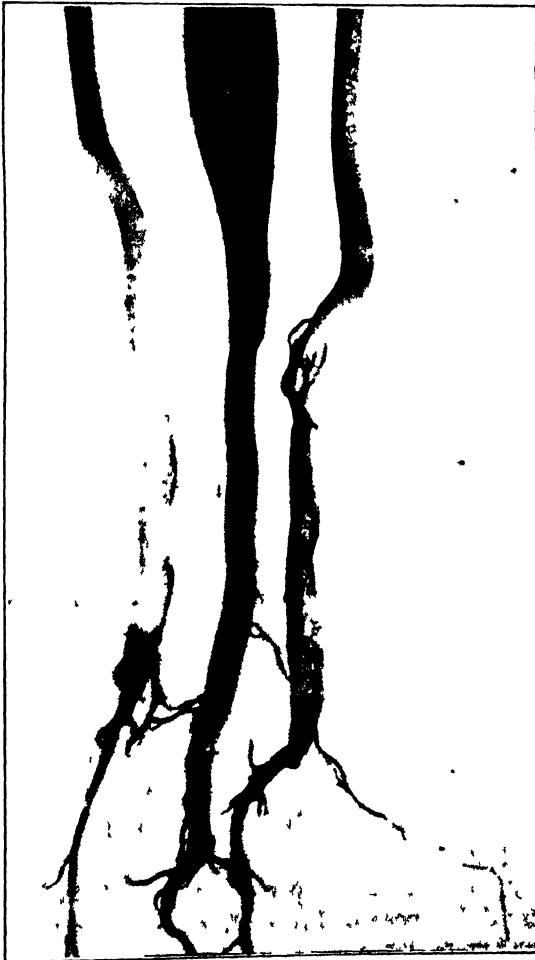


FIG. 9. Typical *Rhizoctonia* diseased seedlings from Flat 2 of figure 8.

The same explanation is probably applicable in the case of the various isolations made in the preceding experiments. In spite of the high infestation of seeds by *Phoma betae*, only a few cultures were obtained. It seems probable that seedlings affected with *Phoma betae*, in large part, under the greenhouse conditions, did not appear above the surface.

In one flat, cornmeal alone was added to the sand to determine the effect of adding this organic nutrient. The total number of seedlings was reduced below that in the sterile sand and the number of diseased seedlings was increased. It seems plausible to attribute these results to some influence of the cornmeal medium upon the organisms present on the seed balls.

The sterile sand gave results in harmony with the previous test on sand. *Phoma betae* was isolated from a diseased seedling.

FIELD TESTS

Since 1922 field tests with various types of treatment of sugar beet seed have been carried out. These tests have, for the most part, been unsatisfactory from the point of view of showing marked results from treatment. The results, although meager and inconclusive, are detailed since they indicate the nature of the problem confronted in the field.

In 1922, commercial sugar beet seed samples were obtained from three companies and each lot divided into three portions. One part of each lot was dusted with copper carbonate, 18 per cent, and one with copper sulphate-lime dust. A third sample of each seed lot was left untreated. Plantings of the nine samples were made at Michigan Agricultural College and there were no differences in stand between the treated and untreated. Similar reports were obtained from Bay City and Saginaw from the cooperating commercial companies. Seed sent to St. Louis, Michigan, was planted by the Holland-St. Louis Sugar Company in rich black soil in which root rot had always been prevalent. These were reported as showing marked differences in stand, and the treated rows stood out all through the season, both because of uniformity of the stand and size of the beets. Unfortunately, yields were not taken. In 1923, more extensive experiments were carried out in which rows of treated seed were compared with rows of untreated seed. Larger plantings were also made of treated seed. Table 9, giving the estimated stand, shows the results of the test. In both cases the soil used had not grown beets recently and the conditions for germination of the seed were exceptionally favorable.

It will be seen that in only one planting, the Schreiber seed at East Lansing, were any differences in favor of treatment to be noted, and these were not so marked as to influence final stand of the beets. The late planting and the influence of the season evidently prevented seedling diseases playing any especial rôle in reducing stand.

A greenhouse planting of the same seed showed results with both sand and soil entirely consistent with former results in the greenhouse. In this test the average stand of the untreated American-grown seed in infested soil was 24 per cent of the assumed normal, and in clean sand it was 27 per cent. Chlorophol and copper carbonate treatments increased the stand. In sand, the final stand was 27 per cent of the assumed normal, and Chlorophol and copper carbonate nearly doubled the stand. Similar results were obtained with Schreiber seed, but the average stand of the Schreiber seed was slightly lower than the American grown seed.

In a field test at St. Louis, Michigan, on a 16-row plot, 37 rods long, different foreign varieties were compared with treated and untreated American grown seed, and the highest tonnage was obtained from treated American grown seed. The results are given in table 10.

In 1924 further comparisons of the treated and untreated seed were made and 3 fields were planted, half to treated and half to untreated seed. On two of the farms no differences in the treated and untreated portions could

TABLE 9.—*Field tests of sugar beet seed treatments at East Lansing and St. Louis Mich. Single rows planted with hand planter. Planted at East Lansing, June 7, 1923; stand estimated June 15, 1923. Planted at St. Louis June 2, 1923; stand estimated June 22, 1923*

Treatment	Stand of Schreiber seed in per cent		Stand of American grown seed in per cent	
	E. Lansing	St. Louis	E. Lansing	St. Louis
Untreated	80	93	100	94
Copper sulphate-lime dust, 6 oz. per 15 lbs. seed	98	98	80	98
Copper carbonate, 18 per cent, dust, 4 oz. per 15 lbs. seed	100	95	80	96
Untreated	70	96	100	92
Copper carbonate, 18 per cent, dust, 6 oz. per 15 lbs. seed	100	96	80	98
Copper carbonate, 18 per cent, dust, in excess	80	96	95	98
Untreated	60	94	80	95
Sulphur; in excess	30	92	80	95
Formaldehyde, 1-240; 30 minutes soaking	98	94	100	92
Untreated	70	94	100	92
Chlorophol, 0.25 per cent; 1 hour soaking	100	95	100	95
Semesan; dust, 4 oz. per 15 lbs. seed	70	95	90	92
Untreated	100	95	100	92

TABLE 10.—*Field tests of treated and untreated sugar beet seed, with standard varieties for comparison: plots consisting of 16 rows, 37 rods long (approximately 0.4 acre), St. Louis, Michigan,^a 1923*

Variety and treatment	Yield per plot in pounds	Sugar percentage
Schreiber and Sohn sugar-beet seed.....	10,640	15.5–15.5
Home-grown seed treated with copper carbonate, 18 per cent, 4 oz. per 15 lb. seed.....	11,260	14.4–15.1
Home-grown seed untreated.....	8,465	14.0–15.1
Danish-grown Kleinwanzlebener	10,300	15.2–15.7
American Beet Sugar Co., leaf-spot resistant seed.....	9,305	14.6–15.4
Delitzcher	9,005	15.0–15.6

^a Figures through courtesy of Mr. B. C. Hubbard, manager of Holland-St. Louis Sugar Co., St. Louis, Michigan.

TABLE 11.—*Field tests^a of various sugar beet seed treatments at East Lansing and St. Louis, Michigan, in 1924. Summary of estimates on per cent of stand made before thinning*

Treatment	Stand obtained in per cent	
	East Lansing	St. Louis
Semesan dust	97.5	100
Mercury bichloride, 1–1,000, 1 hr. soaking.....	95.5	90
Copper carbonate, 50 per cent, dust	94.0	90
Dupont Semesan, No. 13, dust	85.0	95
Uspulun, 0.25 per cent; 1 hr. soaking	85.0	70
Uspulun, 0.25 per cent; 60° C. for 20 minutes	83.0	60
Semesan, 0.25 per cent; 60° C. for 20 minutes.....	83.0	60
Copper sulphate-lime dust	82.5	70
Copper carbonate, 18 per cent, dust	80.0	85
Mercury bichloride, 1–1,000; 60° C. for 20 minutes.....	77.0	80
Small seed balls	69.0	80
Large seed balls	62.5	85
Pasteurization; 60° C. for 20 minutes.....	50.0	50
Formaldehyde, 1–240; 60° C. for 20 minutes.....	40.0	50
Average of checks	75.0	70

^a Test carried on in quadruplicate (two dates of planting). Checks were used for every third row. Plan of experiment and types of treatment at Michigan Experiment Station, East Lansing, are shown in table 12. St. Louis tests were made on farm of Mr. Frank McLean.

be seen. On the third farm a field in which beets had always been unprofitable because of root rot and poor stand was planted partly with seed treated with copper carbonate, partly with seed treated with Semesan, and partly with untreated seed from the same sack as check. The Semesan and copper carbonate gave full stands, with Semesan slightly better than the copper carbonate. The untreated seed gave about two-thirds of a stand.

Single row plots in duplicate, with untreated seed every third row, were planted at St. Louis, Michigan, and at East Lansing. The plan of the experiment may be seen from table 11. Estimation of stand was made approximately two weeks after planting at both East Lansing and St. Louis.

Owing to damage by flooding, the field at St. Louis was not carried through to harvest, but the East Lansing plots were harvested and the results are given in table 12.

In the spring the Semesan dust plots at both East Lansing and St. Louis were best, with other mercury compounds and copper carbonate showing marked superiority over the untreated seed. Attempts to pasteurize seed and to combine pasteurization with a mercury treatment were not successful. Formaldehyde in all cases was the poorest of treatments. The copper dusts gave promise of giving a fair protection, ranking second to the mercury treatments.

The harvest records from the plots showed that the mercury bichloride treatment was first in respect to number of beets and total weight produced. Semesan and Du Pont Semesan 13 were approximately the same and approached the mercury bichloride treatment in effectiveness. The apparent contradiction with the earlier stand record can probably be attributed to the retarding effect that mercury bichloride has upon seedlings, delaying germination and checking growth at the outset. Copper carbonate, 50 per cent, ranked next to the mercury treatments and was better than the untreated seed in both numbers and weight.

DISCUSSION OF RESULTS

The writers believe the field tests just described to be in harmony with the previously outlined greenhouse tests and to demonstrate the applicability of these greenhouse tests to the determination of suitable seed treatments for sugar beets. But we do not believe that the practicability of seed treatment has been shown as yet.

On the other hand, we believe the experiences we have had in several years to be entirely typical of what is to be expected in seed treatments carried out as a commercial practice in Michigan. Damping off of seedlings does not occur in every field or in every season. In about one field in three or four we may expect to demonstrate some marked benefit from seed treatment. Of course in certain seasons, and with certain planting periods,

TABLE 12.—*Field tests with various sugar beet seed treatments^a at East Lansing, Mich. Schreiber and Sohn seed planted in single rows in 25-foot plots, in quadruplicate*

Treatment	Number of beets per plot at end of test					Weight of beets in pounds					Rank based upon	
	Averages				Averages	A	B	C	D	Averages	Number	Weight
	A	B	C	D								
Untreated	14	12	18	16	15.00	17.50	20.00	28.50	21.00	21.75	-	-
Semesan, dust, 6 oz. per 15 lbs. seed	21	21	18	22	20.50	22.25	26.00	32.50	23.00	25.94	2	3
Dupont Semesan 13, dust, 6 oz. per 15 lbs. seed	15	18	20	20	18.25	20.75	23.50	28.75	31.00	26.00	3	2
Untreated	9	16	24	13	15.50	15.50	18.25	28.25	18.50	20.12	-	-
Large seed, untreated	14	14	16	14	14.50	20.75	21.25	21.75	20.00	20.94	-	-
Small seeds, untreated	13	17	16	22	17.00	17.50	23.50	25.00	25.00	22.75	-	-
Untreated	11	20	15	18	16.00	14.00	25.50	24.75	23.75	22.00	-	-
Copper carbonate, 18 per cent, dust	11	20	7	19	14.25	14.75	24.50	11.00	23.00	18.31	-	-
Copper carbonate, 50 per cent, dust	10	22	21	20	18.25	17.50	28.00	32.25	26.00	25.94	4	4
Untreated	11	15	15	13	13.50	23.00	19.25	32.00	26.00	24.06	-	-
Pasteurized, 60° C. for 20 minutes	2	20	6	15	10.75	5.25	27.00	16.25	24.50	18.25	-	-
Formaldehyde, 1-240; 60° C. for 20 minutes	2	20	12	8	10.50	4.50	28.00	23.25	11.25	14.75	-	-
Untreated	11	22	18	18	17.25	16.00	29.00	34.75	29.00	27.19	-	-
Mercury bichloride, 1-1000; 60° C. for 20 minutes	10	20	1	18	12.25	13.75	25.25	2.00	22.50	15.81	-	-
Mercury bichloride, 1-1000; 1 hour soaking, room temperature	25	21	22	24	23.00	28.75	21.25	45.50	28.00	30.88	1	1

TABLE 12.—Continued

TABLE 12.—Continued

Treatment	Number of beets per plot at end of test					Weight of beets in pounds					Rank based upon	
	Averages					Averages					Number	Weight
	A	B	C	D	Averages	A	B	C	D	Averages		
Untreated	7	25	21	15	17.00	11.50	29.00	30.75	19.00	22.31	—	—
Uspulun, 0.25 per cent; soaking 1 hour, room temperature	14	20	20	16	17.50	21.75	24.00	31.75	22.00	24.88	—	—
Copper sulphate-lime dust, 6 oz. per 15 lbs. seed	8	17	18	16	14.75	16.25	26.00	28.50	15.50	21.56	—	—
Untreated	10	18	21	15	16.00	19.50	23.25	32.00	19.50	23.56	—	—
Uspulun, 0.25 per cent; 60° C. for 20 minutes	11	20	18	12	15.25	19.00	22.50	28.25	14.00	20.93	—	—
Semesan, 0.25 per cent; 60° C. for 20 minutes	14	20	23	13	17.25	27.00	20.75	31.00	17.75	24.12	—	—
Untreated	5	—	19	11	11.66	17.00	—	30.50	17.50	21.66	—	—
Untreated	7	21	18	12	14.50	17.50	28.25	31.00	12.50	22.31	—	—
Average of untreated rows	15.2 ± 0.39					22.70 ± 0.55						

Average of untreated rows ... the concentration of corner carbonate, 50 per cent, which was material prepared for cereal dusting

^a Chemicals used were the same as given in tables with the exception of copper carbonate, 50 per cent, which was material prepared for cereal dusting by Riches and Piver, New York City.

The dusts were applied by churning in a barrel for 2 minutes approximately 10 pounds of seed and the requisite amount of dust. The seed was placed in thin cloth bags, loosely tied, and immersed in the water. Temperature readings were taken within the bags. The seed was dried over night by being spread out in thin layers.

damping off is very serious and then the gain from treatment would be large. Furthermore, certain of the seed treatments employed are entirely too costly at present prices of the chemicals. The soaking treatments seem to be completely precluded from practical use because of the difficulty of drying the seed balls in large quantities at the seed warehouses. When it is remembered that for the ordinary acreage of a factory nearly 100,000 pounds of seed would be handled, the impracticability of a wet treatment is obvious.

The writers believe that the application of dusting of sugar beet seeds with suitable disinfectants has great promise for the sugar beet industry. It seems that it is possible by the use of disinfectants to prevent in large part the serious reduction of stand which arises from the seed-borne pathogens. What is still more significant is the action of these disinfectants in actually warding off the pathogens so common in the soil, thus allowing the seedling to become established. If our opinion, that the root rots which develop late in the growing crop are the result of diseases contracted in the seedling stage, is correct, seed treatment of sugar beets may be of great value in preventing root rot losses.

While our experiments have not progressed far enough to permit recommendation of any compounds for general use, it is believed that progress has been made. The copper compounds we have tried do not seem effective enough to give the protection desired, and it seems safe to predict that some combination of mercury compounds with copper dusts will more nearly meet the need of a fairly cheap, effective dust for seed treatment.

SUMMARY

Seedling diseases of sugar beets, although often overlooked, cause serious losses in many sections. These seedling diseases are looked upon as especially important because of their relation to the diseases known as root rot, which develop on the half-grown and mature beets. Previous work has shown that *Phoma betae*, *Pythium debaryanum* and other Phycomycetes as well as *Rhizoctonia* spp. are the principal organisms causing seedling diseases of sugar beets in Europe and America. No practical control measures have been developed.

The seedling diseases studied have fallen into three general types, but assignment by field examination to a definite organism is hazardous.

Seed grown from carefully selected roots, kept under excellent storage conditions, and harvested by hand after growth in isolated plots, was found to be infested with *Phoma*, confirming the reports in the literature of the almost universal infestation of beet seed with this organism.

Greenhouse tests with muck soil and sandy loam soil showed that germination is reduced enormously by the beet pathogens and this reduction of stand increases if the soil is used repeatedly. Besides the loss before the

seedling emerges from the soil, there is a progressive loss in stand by damping-off of the seedlings during the period in which the true leaves are developing.

Pythium debaryanum was found to be the most common organism associated with diseased seedlings on muck soil, and it also was very prevalent in sandy loam soil. *Phoma betae* and *Rhizoctonia* were also isolated.

Tests with seedlings grown in sand showed that 33 per cent of the seedlings appearing above the surface of the sand may be killed by damping-off, *Phoma betae* being chiefly responsible, *Pythium* appearing but seldom. Ordinary plating methods may not indicate faithfully the etiological factors with diseases of this type.

Pasteurization at 60° C. for 10 minutes on two successive days proved to be a very effective treatment when pathogens of a soil source were absent, but of no value in infested soil, indicating that not only *Phoma betae* must be considered, but that *Pythium* and other organisms play their rôles in the damping-off complex.

Inoculation experiments showed that *Phoma betae* and *Pythium debaryanum* were both very strong and rapidly working parasites. *Rhizoctonia* sp. causes a high percentage of disease but is slower in producing its effects and leads to partially affected plants. Seed treatment was not effective with wholesale soil infestation.

The fungus content of the substratum and of the seed ball, rather than the physical nature of the soil, seemed to be the decisive factors in the incidence of disease.

Formaldehyde, furfural, nickel carbonate dust, and the proprietary compounds, Kalimat, Tillantin B, and Seed-O-San, were of little or no value under the conditions of our tests.

Pasteurization at 60° C. for 10 minutes, one day, or on two successive days, and dry heat, 93° C. or 110° C. for 10 minutes, failed to give protection with infested soil.

All of the tests are consistent in showing that mercury and copper compounds not only reduce the diseases arising from a seed source, but have more or less value in a protective action against diseases arising from the soil.

Semesan as a dust in excess and Dupont Semesan 13 in excess have been the leading dust treatments in greenhouse tests; and Chlorophol, 1-400; Uspulun, 1-400; and mercury bichloride, 1-1000, have given strong reduction of disease and stands far superior to the untreated seed.

Copper carbonate, 18 per cent, and copper sulphate-lime (50-50) dusts have given consistently fair control of disease and fair stands.

Field results have not been especially convincing, largely because favorable growing conditions have given good stands in the untreated plots in

spite of the disease factors. The general results indicate that the conclusions from the greenhouse experiments will be duplicated in the field tests.

Wet treatments are impractical for commercial use because of the difficulties inherent in drying the seed in quantity. The mercury treatments seem too costly to be practical. It is suggested that, since the dusts containing copper compounds have fair fungicidal value and are cheap enough to be practical, it may be possible to combine a copper dust with dusts containing mercury to obtain a cheap disinfectant having commercial possibilities.

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SULPHUR AS A CONTROL AGENT FOR COMMON SCAB OF POTATO

G. H. DUFF AND CATHERINE GRAHAM WELCH

In recent years efforts have been made to bring various pathogenic soil organisms under control by employing fungicidal materials as soil dressings. Among these materials sulphur has played a conspicuous role, particularly as a prospective agent for the control of common scab (actinomycosis) of potato. Since destructive diseases of this important crop have given indications of being amenable to sulphur treatment, the importance of a fuller understanding of the potentialities of this substance has not been lost on the industry. Generous support has therefore been forthcoming for the investigation of sulphur as a fungicide, and a fellowship for this purpose was allocated to one of the authors in 1922 by the Special Sulphur Fellowships Committee of the Division of Biology and Agriculture, National Research Council. To this Committee and to the Texas Gulf Sulphur Company of New York, the donor of the Fellowship, the authors are deeply indebted, not only for the opportunity afforded to study this important problem, but also for their cordial and helpful co-operation in many ways.

At the time this work was projected, very promising results had already been obtained by Martin (5) in New Jersey. The preliminary indications from Martin's work were that the incidence of scab could be considerably reduced by treating the soil with sulphur, and further that this control was brought about by acidification of the soil following the oxidation of the sulphur thus applied. The second conclusion appeared to be a confirmation of the work of Gillespie and Hurst (3) and of Gillespie (2) who found in the first place a correlation between soil acidity and the distribution of the potato scab organism in the field, and in the second place, that artificial culture media at or below pH 5.0 were very much less favorable for the cultivation of the organism than those of higher exponents. It appeared to these workers, therefore, that the control of the organism by sulphur dressings was dependent simply upon the attainment of a degree of acidity sufficient to inhibit its growth. This in turn is obviously dependent on the effective oxidation of the sulphur applied; and since the oxidation of sulphur in the soil has been shown by Lipman and his associates (4) to be mainly a biological process, there arose the expectation that sulphur inoculated with *Thiobacillus thiooxidans* would prove to be more effective as a control agent

than ordinary flowers of sulphur. This expectation seemed to be realized in the results of subsequent experiments by Martin (5).

The present authors felt that if these highly favorable and encouraging conclusions could be confirmed over a wide geographical area, a valuable contribution would have been made. Two major questions were envisaged:

1. Is sulphur equally effective in controlling potato scab on all soils and under all climatic conditions?
2. Is the lethal effect of sulphur upon *Actinomyces* always dependent solely, or even for the most part, upon the increased acidity following its oxidation in the soil?

A question of minor importance which was kept in view concerned the relative values of flowers of sulphur and of inoculated sulphur as control agents. This is obviously bound up with the second of the two major questions above.

In order to find answers to these questions, it was clearly necessary to conduct field trials with inoculated sulphur and with flowers of sulphur upon a number of soils varying as to type and history and well scattered geographically. It would have been quite impossible to do this had it not been for the cooperation of the Division of Botany, Experimental Farms Branch, Canadian Federal Department of Agriculture, the Ontario Agricultural College, and the Manitoba Agricultural College. Thanks to the admirable manner in which these institutions responded to our request, it was possible to conduct ten experiments in 1923 in important potato growing regions of Prince Edward Island, Nova Scotia, New Brunswick, Ontario, Manitoba, and Saskatchewan. One of these is not reported since the reliability of the results was made doubtful by irregularities in experimentation.

FIELD OPERATIONS

It was obviously impossible for the authors to give direct supervision to the field operations of every experiment. With the exception of the experiment at Guelph, which was thus supervised, each one was under the direction of some member of the staff in plant pathology of the Dominion Department of Agriculture or one of the agricultural colleges. Full and identical directions concerning the preparation of the land, the application of sulphur, planting, sampling of soils, making of notes, and lifting and classifying the crop were sent out to each of those concerned, in an effort to ensure uniformity of procedure. These directions were simplified in so far as possible in order to minimize the difficulty of adhering strictly to them. The authors were informed whenever, for any reason, the procedure deviated from that laid down in the directions. Towards the close of the season one of the authors made a tour of inspection, which included all the less remote

stations. This served as an assurance that the various field operations were being conducted in a manner that justified the comparison of results.

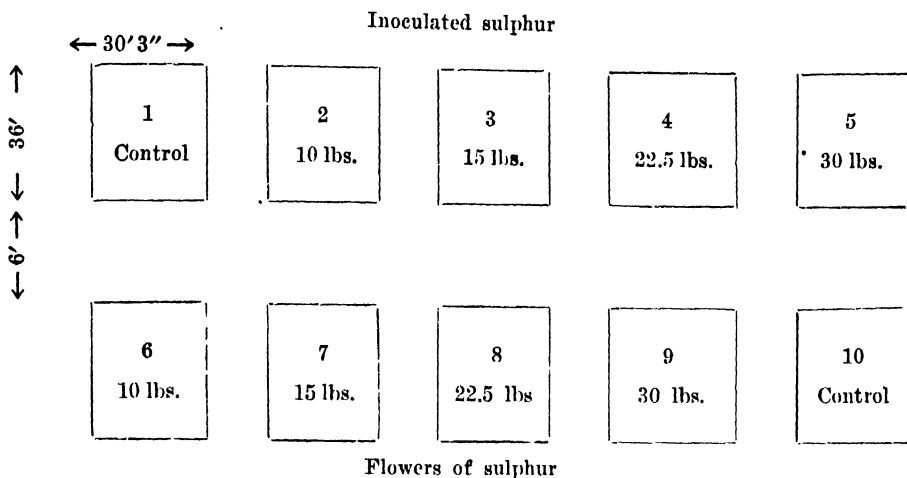


FIG. 1. Diagram of experimental plots.

Each field experiment consisted of ten plots, 1/40 acre in area, laid out and treated as shown in figure 1. The ground in each case was first worked up and the plots squared off. The first series of soil samples was then taken. The sulphur was broadcast immediately afterwards and raked in thoroughly, after which the potatoes were planted. The seed potatoes used were certified Irish Cobblers. These were cut as uniformly as possible and planted at a depth of 4 inches. The sets were separated in the rows by 15 inches and the rows themselves were 36 inches apart. In each plot 22½ lbs. of potatoes were planted, which is equivalent to a proportion of ten bags per acre.

The details of cultivation and of the employment of fungicides and insecticides were necessarily left to the officer in charge of the station, who employed the measures which experience had shown to be the most effective in his own locality. In point of fact these were not seriously different as between station and station, and such small differences in practice as there were do not invalidate the comparisons which will be made.

Soil samples were taken periodically and the sampling dates are, for the most part, shown in the tables. Six well-scattered samples were taken from the rows in each plot, the six mixed thoroughly and a final sample of about 250 grams taken from the mixture to represent the plot. These plot samples were at once put into separate cardboard cartons together with an identification card upon which were recorded notes concerning the growth of the crop, the incidence of diseases of the vines, the state of cultivation, etc.,

for the interval following the taking of the previous sample. The cartons were immediately posted to Toronto, where the acidity determinations were made.

At the end of the season the potatoes were dug and those from each plot sorted separately into three classes. Class I consisted of potatoes wholly free from common scab. In Class II were placed potatoes which were defaced by no more than two fair-sized scab lesions. Such tubers, while inferior, were regarded as bearing a market value for table use. In Class III were placed all those tubers which were more heavily infected than those of Class II. The presence or absence of diseases other than scab played no part in the classification, although notes were made of them during the process of grading. Subsequently the proportion of the total yield falling into each class was expressed as a percentage of the total yield.

ACIDITY DETERMINATIONS

The soil samples, received periodically in lots of ten, from each station, were subjected to analysis with as little delay as possible. The soil was at once air-dried and then passed through a sieve of .01 sq. mm. mesh. Fifteen grams of this sifted soil was then weighed into a centrifuge tube and 30 cc. of distilled water added to it. The tube was then shaken 75 times, after which the extract was cleared by centrifuging for five minutes at about 2500 r. p. m. Five cc. of the supernatant liquid were withdrawn into a narrow-bore test tube, five drops of indicator added and matched in a comparator against color standards of 0.2 pH intervals.

A few of the earlier acidity determinations were made from extracts which had been cleared by standing for 4 to 6 hours. When the centrifuge became available the two methods of clearing were compared upon the same soil samples, and the acidities resulting were found to check consistently. Thereafter the extracts were cleared exclusively by centrifugation.

For the purposes of the color standards McIlvaine's (1, p. 116) buffer mixtures were employed. These were carefully checked at frequent intervals by the hydrogen electrode, and renewed whenever necessary. The indicators used were those of Clark and Lubs (1, p. 80).

TABULATION OF RESULTS

Tables 1 to 9 summarize the results of the nine field experiments of 1923. Each table shows the ten plots involved in the experiment, their respective sulphur dressings, the total yield resulting, which is classified according to classes, and a record of the soil acidity throughout the season. Brief notes are given on the recent history of the soil with respect to lime and fertilizer dressings and to crops. Notes on the progress of growth and

TABLE 1.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Bedford, Prince Edward Island, in a sandy loam with red brick clay subsoil^a

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples					
	Type	Pounds per acre		I	II	III	May	June	July	Aug.	Early Sept.	Late Sept.
1	Inoculated sulphur	0	151	10.6	48.3	41.1	6.5	6.0	6.0	6.0	6.0	6.0
2		400	142	10.5	45.8	43.7	6.0	6.2	5.8	5.8	5.8	5.9
3		600	133	3.1	41.3	55.6	6.3	6.1	6.0	6.0	6.0	5.4
4		900	143	13.9	50.4	35.7	6.3	6.0	5.6	5.8	5.8	5.8
5		1200	132	9.4	34.4	56.2	6.0	6.1	5.2	5.7	6.0	5.3
6	Flowers of sulphur	400	143.0	8.5	46.8	44.7	6.0	6.2	6.0	6.0	6.0	5.8
7		600	151.0	3.9	45.6	50.5	6.2	6.2	5.8	5.2	5.8	5.8
8		900	166.5	2.4	40.5	57.1	6.4	6.3	5.5	5.7	5.7	5.9
9		1200	157.5	3.8	37.7	58.5	6.5	6.1	6.0	5.5	5.6	5.3
10		0	144.0	0.0	4.2	95.8	6.2	6.4	6.0	5.5	6.2	6.4

^a In 1917 mussel mud (80–85 per cent lime) was applied to the soil at the rate of 10 tons per acre. The land was in potatoes in 1921 and scab was severe. In 1922 barley and clover were planted.

^b Field notes: Growth was never better than fair during a late season. Blackleg appeared relatively early and was fairly uniformly destructive.

Rainfall.

June 2–23	1.28 inches	Aug. 2–Aug. 24	2.00 inches
June 23–July 13	2.75 do	Aug. 24–Sept. 20	3.95 do
July 13–Aug. 2	1.69 do	Weekly mean	0.71 do

TABLE 2.—*The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at New Mills, Restigouche County, New Brunswick, in a sandy loam^a*

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples					
	Type	Pounds per acre		I	II	III	May 30	June 20	July 19	Aug. 17	Sept. 5	Sept. 24
1	Inoculated sulphur	0	167.5	0.0	45.7	54.8	5.1	5.2	5.2	5.2	5.0	5.0
2		400	170.5	1.5	72.1	26.4	5.4	5.3	5.2	5.0	5.1	4.9
3		600	190.5	13.9	78.7	7.4	5.4	5.2	5.0	4.5	4.4	4.9
4		900	187.5	20.4	76.9	2.7	5.4	5.4	4.6	4.6	4.5	4.7
5		1200	159.5	6.9	82.2	10.9	5.4	5.8	4.6	4.5	4.4	4.6
6	Flowers of sulphur	400	88.5	20.3	64.5	15.2	5.1	5.0	4.8	4.4	4.6	4.6
7		600	120.5	12.2	65.9	21.9	5.4	5.4	5.0	4.4	4.6	4.7
8		900	153.5	9.1	76.8	14.1	5.4	5.4	4.7	4.7	5.0	4.5
9		1200	139.5	18.6	80.3	1.1	5.4	5.4	5.0	4.4	4.6	4.4
10		0	159.0	19.5	63.6	16.9	5.4	5.4	5.4	5.3	5.2	5.3

^a In 1917 the soil was limed with fish and seaweed (200 pounds per acre) and subsequently has been dressed frequently with lobster, clam shells, and other calcareous material. The land was in potatoes for several years. Scab was invariably present and was severe in 1922.

^b *Field notes:* Growth was fair, with a few misses and a small number of plants developing mosaic, leafroll, and blackleg. Plots 1-5 seemed to get a better start than plots 6-10 and contained fewer weak plants. This initial superiority was maintained and reflected in the yield. Tubers showed traces of rhizoctonia, blackleg, and silver scurf.

TABLE 3.—*The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Sackville, New Brunswick, in a sandy loam^a*

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples						
	Type	Pounds per acre		I	II	III	May 29	June 19	July 14	Aug. 6	Aug. 28	Sept. 13	
1	Inoculated sulphur	0	265	26.4	65.3	8.3	5.6	5.2	5.6	6.0	5.6	6.0	
2		400	322	24.8	71.8	3.4	5.6	5.0	4.9	5.6	6.4	4.7	
3		600	377	26.2	72.9	0.9	5.5	4.9	4.6	4.8	5.0	4.4	
4		900	362	21.8	75.9	2.3	5.2	4.4	4.0	4.0	4.6	3.7	
5		1200	342	13.7	78.2	8.1	5.1	5.2	4.0	4.0	3.8	4.0	
6	Flowers of sulphur	400	415	20.2	78.5	1.3	5.2	5.1	5.2	5.2	5.0	5.0	
7		600	368	20.7	77.9	1.4	5.4	5.6	5.0	4.6	4.9	4.9	
8		900	468	6.8	85.7	7.5	5.1	5.0	4.0	4.6	4.6	4.8	
9		1200	397	3.3	81.3	15.4	5.0	4.6	4.0	4.2	4.0	4.4	
10		0	378	10.1	79.6	10.3	5.0	4.8	5.3	5.2	5.3	5.2	

^a The land was in sod for 7 years prior to 1921, and had never been limed. In 1921 and 1922 the potato crop was unfit for the market on account of scab.

^b *Field notes:* Plants were vigorous and healthy during most of the growing season, although slightly affected by late blight. Tubers showed traces of late blight and blackleg.

Rainfall.

May 29–June 19	1.26 inches	Aug. 6–Aug. 28	1.18 inches
June 19–July 14	2.99 do	Aug. 28–Sept. 13	0.35 do
July 14–Aug. 6	2.62 do	Weekly mean	0.53 do

TABLE 4.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Plaster Rock, Victoria County, Nova Scotia, in a sandy loam^a

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples						
	Type	Pounds per acre		I	II	III	May 29	June 19	July 10	July 31	Aug. 21	Sept. 28	
1	Inoculated sulphur	0	405.0	96.2	2.5	1.3	5.0	5.4	5.6	5.4	5.2	4.9	
2		400	310.0	98.0	1.6	0.4	5.1	5.3	5.4	5.2	5.2	4.4	
3		600	330.0	97.8	1.3	0.9	5.2	5.2	5.0	4.8	4.4	3.5	
4		900	295.5	99.0	0.4	0.6	5.0	5.2	5.0	4.8	4.0	3.6	
5		1200	265.0	99.6	0.4	0.0	5.4	5.0	4.6	4.8	3.8	3.4	
6	Flowers of Sulphur	400	510.0	98.4	0.6	1.9	5.4	5.5	5.2	5.1	5.2	4.7	
7		600	360.0	94.6	4.2	1.2	5.1	5.3	5.2	5.1	5.0	4.8	
8		900	425.0	97.5	1.2	1.3	5.2	5.4	5.0	5.2	5.1	4.6	
9		1200	390.5	95.5	3.6	0.9	5.0	5.3	5.0	5.2	5.0	4.5	
10		0	273.0	97.0	1.8	1.2	5.1	5.3	5.4	5.4	5.2	5.2	

^a The land was in hay for several years prior to 1923 and had never been limed.

^b Rainfall.

May 29–June 19	0.10 inches	July 31–Aug. 21	1.33 inches
June 19–July 10	1.58 do	Aug. 21–Sept. 28	2.52 do
July 10–July 31	3.13 do	Weekly mean	0.49 do

TABLE 5.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Central Experimental Farm, Ottawa, Ontario, in a clay loam^a

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples				
	Type	Pounds per acre		I	II	III	June 1	June 21	July 14	July 31	Aug. 27
1	Inoculated sulphur	0	386	99.1	0.9	0	6.2	6.0	5.8	6.1	6.2
2		400	450	98.1	1.9	0	6.2	6.0	6.0	6.0	6.0
3		600	496	98.8	1.2	0	6.1	6.0	5.9	5.3	5.8
4		900	549	96.3	3.7	0	6.2	6.0	6.0	5.5	5.2
5		1200	585	97.6	2.4	0	6.2	5.9	6.0	5.0	5.0
6	Flowers of sulphur	400	376	98.5	1.5	0	6.1	5.9	5.5	5.7	5.6
7		600	272	98.9	1.1	0	6.0	6.0	5.6	5.6	5.4
8		900	486	96.8	3.1	0.1	6.2	5.8	5.9	5.5	5.0
9		1200	495	88.7	11.3	0	6.2	5.6	6.0	5.0	4.9
10		0	535	97.1	2.9	0	6.2	6.0	6.0	6.0	6.0

^a Prior to 1923 a variety of crops had been grown: grass, oats, buckwheat, and truck crops.

^b Field notes: Growth was good on the whole, but there were misses, on account of excessive soil moisture, which were sufficiently serious in number to affect the figures for yield. Leaf roll present, but in only two or three plants.

Rain/fall:

June 1-June 21	3.42 inches	Aug. 6-Aug. 27	1.99 inches
June 21-July 14	3.53 do	Aug. 27-Sept. 17	3.42 do
July 14-Aug. 6	1.93 do	Weekly mean	0.95 do

TABLE 6.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Ontario Agricultural College, Guelph, Ontario, in a clay loam^a

Plot no.	Sulphur		Total yield in lbs. b	Yield in each class in per cent			pH of soil samples						
	Type	Pounds per acre		I	II	III	May 25	June 16	July 7	July 28	Aug. 18	Sept. 8	
1	Inoculated sulphur	0	166.0	0	0.4	99.6	7.2	7.4	7.0	7.0	7.0	7.0	
2		300	162.5	0	6.5	93.5	7.2	7.1	6.6	6.8	6.9		
3		600	168.0	0	6.9	93.1	7.2	7.0	6.7	6.6	6.8		
4		900	168.0	0	4.8	95.2	7.2	6.7	6.5	6.6	6.6		
5		1200	162.5	0	4.9	95.1	7.2	7.1	6.6	6.6	7.0		
6	Flowers of sulphur	300	101.5	0.6	28.5	70.9	7.2	7.2	7.2	7.0	7.0		
7		600	142.5	0.3	19.6	79.6	7.2	7.0	7.0	6.8	7.0		
8		900	138.0	0.2	12.3	87.5	7.2	6.7	6.5	6.8	6.8		
9		1200	141.5	0.6	27.2	72.2	7.2	6.8	6.3	6.2	6.4		
10		0	166.5	0.0	2.2	97.8	7.2	7.5	6.8	7.1	7.0		

^a The soil had never been limed. In 1920 the potato crop was slightly affected by scab. The land was in clover and mangels in 1921 and 1922.

^b Field notes: Growth of the plants was uniformly good. The only disease appearing was tip-burn which was noted first in the middle of August. Rhizoctonia present at digging.

Rainfall:

May	4.00 inches	Aug.	4.86 inches
June	4.46 do	Sept.	3.14 do
July	3.11 do	Weekly mean	0.91 do

TABLE 7.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Devlin, Rainy River District, Ontario, in a heavy clay^a

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples					
	Type	Pounds per acre		I	II	III	May 22	June 18	July 10	July 31	Aug. 21	
1	Inoculated sulphur	0	560	18.1	81.9	0	7.4	7.4	7.4	7.4	7.4	
2		400	554	31.1	68.9	0	7.4	7.4	7.2	7.3	7.4	
3		600	558	50.6	49.4	0	7.4	7.2	7.0	7.2	7.0	
4		900	543	75.1	24.9	0	7.4	7.4	7.0	7.1	7.2	
5		1200	547	69.1	30.9	0	7.4	7.6	7.0	7.0	6.7	
6	Flowers of sulphur	400	538	69.6	30.4	0	7.4	7.4	7.0	7.2	7.0	
7		600	579	83.2	16.8	0	7.4	7.3	7.2	7.2	7.3	
8		900	572	73.5	26.5	0	7.4	7.2	6.7	7.2	7.4	
9		1200	563	69.5	30.5	0	7.4	7.2	6.8	7.3	7.2	
10		0	587	33.3	66.7	0	7.4	7.4	7.3	7.4	7.4	

^a No lime or fertilizer has ever been applied to the soil. In 1919 the potato crop was severely affected by scab. The land was in clover in 1920-1922.

^b Field notes: Growth of the plants was uniformly good and they remained free of disease.

Rainfall:

May	0.47 inches	Aug.	1.12 inches
June	2.55 do	Sept.	2.37 do
July	2.94 do	Weekly mean	0.43 do

TABLE 8.—The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Manitoba Agricultural College, Winnipeg, in well-drained, black, Red River Valley clay^a

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples						
	Type	Pounds per acre		I	II	III	May 25	June 13	July 9	July 30	Aug. 4	Sept. 12	
1	Inoculated sulphur	0	156.5	48.3	49.8	1.9	6.6	6.6	6.6	6.6	6.6	6.6	6.4
2		400	163.0	65.3	33.2	1.5	7.3	7.1	6.4	6.0	6.4	6.1	
3		600	86.0	75.5	23.3	1.2	6.6	6.5	6.0	6.0	6.2	5.8	
4		900	55.0	81.8	16.8	1.4	7.4	6.8	6.6	6.2	6.2	5.6	
5		1200	71.0	85.9	14.1	0.0	6.8	6.6	6.0	6.0	6.0	5.4	
6	Flowers of sulphur	400	183.0	60.4	37.9	1.7	6.6	6.6	6.3	6.3	6.6	6.0	
7		600	155.5	59.7	39.9	0.4	6.6	6.3	6.0	6.0	6.7	6.0	
8		900	93.0	79.6	20.4	0.0	6.5	6.0	5.9	6.0	6.1	5.0	
9		1200	110.5	80.9	18.6	0.5	6.8	6.3	5.9	6.0	6.0	5.5	
10		0	136.0	46.3	49.6	4.1	6.6	6.6	6.6	6.6	6.7	6.4	

^a The sod was newly broken in 1921, and has never been limed. Potatoes grown in 1921 were slightly affected by scab. The land was in wheat in 1922.

^b Field notes: Growth throughout the season appeared to be good for the most part, although early dry weather resulted in retardation of individual plants here and there. A small number of plants were affected by leaf roll. Tubers showed rhizoctonia and black scurf.

Rainfall:

May	2.55 inches	Aug.	0.65 inch
June	1.47 do	Sept.	1.09 do
July	3.55 do	Weekly mean	0.42 do

TABLE 9.—*The effect of sulphur on the incidence of common scab on potatoes grown in 1923 at Esthern, Saskatchewan, in a well-drained, black, sandy prairie loam with 6-18 inches of impervious clay subsoil*

Plot no.	Sulphur		Total yield in lbs. ^b	Yield in each class in per cent			pH of soil samples				
	Type	Pounds per acre		I	II	III	June 6	June 28	July 17	Aug. 7	Aug. 29
1	Inoculated sulphur	0	517	8.3	76.9	14.8	6.0	6.0	6.0	6.2	6.0
2		400	538	8.7	77.6	13.7	6.0	6.0	6.0	5.8	5.8
3		600	533	10.2	83.5	6.3	6.0	5.8	5.8	5.8	5.9
4		900	570	12.4	85.3	2.3	6.0	6.0	6.0	5.9	6.0
5		1200	505	22.9	75.7	1.4	6.0	6.1	6.0	5.9	5.8
6	Flowers of sulphur	400	587	13.5	84.8	1.7	6.1	5.8	6.0	6.0	6.0
7		600	537	13.1	72.8	14.1	6.1	5.8	6.0	6.2	6.0
8		900	546	12.5	70.9	16.6	6.1	6.2	6.0	6.2	6.0
9		1200	494	3.1	71.2	25.7	6.0	6.0	6.0	6.0	6.0
10		0	400	4.5	64.3	31.2	6.0	6.0	6.0	6.0	6.0

^a The soil was broken about 1913, and has never been limed. The land was in oats in 1921 and lay fallow in 1922.

^b Field notes: Growth began unevenly and slowly under dry conditions but by the middle of July it was good. Blackleg and rhizotonia appeared early in a few plants and later on there were traces of blight.

Rainfall:

June 6-June 28 2.90 inches
 June 28-July 17 1.95 do
 July 17-Aug. 7 2.87 do

Aug. 7-Aug. 29 1.52 inches
 Weekly mean 0.71 do

the incidence of disease throughout the season, and data on rainfall have been given where these were available.

ANALYSIS OF RESULTS

Attention may now be directed to the questions chiefly under consideration, namely, whether or not sulphur is equally effective in control under various conditions of soil and climate and whether or not the adverse effect of sulphur upon the disease is merely a function of its effect upon the acidity of the soil. To do this it is necessary to decide in which experiments there were indications of satisfactory control and in which ones sulphur applications resulted in significant increases in soil acidity as measured by the hydrogen-ion concentration of watery extracts.

The only results which might be considered to give any sort of indication of successful control are those at Devlin and Winnipeg (Tables 7 and 8), where there were substantial increases in the proportions of clean tubers, and those at New Mills and Sackville (Tables 2 and 3), where the proportions of slightly infected tubers (Class II) were large. In the latter two, however, either one or both control plots produced crops with sufficiently large proportions of Class II tubers to make insignificant the increases shown in the experimental plots. It must be concluded, therefore, that the only experiments which offered unequivocal and satisfactory indications of control were those at Devlin and Winnipeg. In these experiments sulphur applications brought about substantial and roughly proportional increases in the percentages of clean tubers, and with the higher applications led to crops in which the vast majority of the potatoes were free from scab. Nothing less definite than this can be considered a satisfactory or successful result.

Before pointing out any apparent correlations between successful control on one hand and factors of soil and climate on the other, we must first enquire whether factors other than these could have determined the conclusion that the experiments at Winnipeg and Devlin were the only ones successful in control. In the first place the circumstance that the crops at the various experimental centers were not graded by the same person might be supposed to introduce an important source of error. The scheme of grading was very simple, however, dependent at its most critical point (the distinction between potatoes infected and those not infected with scab) solely upon the ability to recognize common scab. Since all the field collaborators were thoroughly familiar with diseases of the potato, it is hardly possible that the conclusion drawn could have resulted from lack of uniformity in grading.

A more serious point is the one very properly emphasized by Roach, Glynne, Brierley, and Crowther (7) in their admirable paper on the control

of potato wart disease, namely, the thoroughness of the incorporation of the sulphur with the soil. It is obvious that the employment of the same procedure in each case will not bring about the same state of incorporation of the sulphur in wholly different soils. Furthermore, in two of the present experiments the method of incorporation differed from that employed in the others. At Guelph the sulphur was worked in by the harrows, and at Winnipeg after planting by hoeing and cultivating. The authors are, therefore, not in a position to say that the sulphur was equally well incorporated in all the experimental plots here compared. Assuming, however, that the more intimate the admixture of sulphur the greater its effect upon the organism, it is interesting to note that the only two experiments in which a satisfactory degree of control was obtained were those wherein, for different reasons, a relatively poor state of incorporation might have been expected. At Winnipeg the method of working in the sulphur can hardly have been as effective and certainly not more so than that employed elsewhere. At Devlin the soil is a heavy and intractable clay into which, using the same method, it would be considerably more difficult to incorporate the sulphur than into a sandy loam soil. Moreover, the great differences in the degree of control attained at Winnipeg and Bedford, for instance (Tables 8 and 1), would appear to postulate improbable differences in the state of incorporation of the sulphur, interpreted on that basis alone.

TABLE 10.—*A summary of the effectiveness of sulphur in controlling common scab on potatoes grown in nine different localities of Canada in 1923*

Location of experiment	Control	Depression of pH	Soil type	Lime	Rainfall index
Plaster Rock...	Organism absent or non-virulent	5.1-3.4	Sandy loam	None	0.49
Ottawa		6.1-4.9	Clay loam	do	0.95
Winnipeg	Positive	6.7-5.4	Clay	None	0.42
Devlin	Positive	7.4-6.7 ^a	Clay	None	0.43
Bedford	Negative	6.2-5.3	Sandy loam	1917	0.71
New Mills	do	5.3-4.4	Sandy loam	1917-1922
Sackville	do	5.4-3.7	Sandy loam	None	0.53
Guelph	Negative	7.1-6.2 ^a	Clay loam	None	0.91
Rosthern	do	6.0-5.8 ^a	Sandy loam	None	0.77

^a An insignificant acidity increase. Figures in the column "Depression of pH" were derived as follows: The first figure is the average of all determinations upon untreated soil, and the second is the lowest single exponent obtained in each experiment. For further details tables 1 to 9 should be consulted.

A third consideration has to do with the prevalence of virulent strains of the scab organism in the soil at the beginning of the experiment. Judging from the percentage of infected tubers in the control plots, this is obviously very different in the various experiments with which we are dealing. It is also clear that in the two experiments most successful in the matter of control, the soil was not so heavily infested as in those which were unsuccessful.

When we come to the factors of soil and climate and their influence in control, two points of interest arise: the first is that both soils which gave an encouraging degree of success were clay soils. The second is that the rainfall was lower at these than at any other experimental points and considerably lower than at most of them.

The considerations under discussion are brought together in table 10, in which the "rainfall index" is the weekly mean rainfall for the season at each experimental point (see tables 1 to 9). Since soil moisture determinations were out of the question and because all the experimental plots were adequately drained, it was felt that this index yielded a convenient, if rough, measure of the moisture factor.

The results, in so far as control is concerned, may be summarized from table 10 in these terms: With the methods here employed, sulphur was not uniformly effective under all conditions of soil and climate, but gave encouraging results only upon clay soils, infested in but moderate numbers or by attenuated strains of the organism and under conditions of low moisture.

Tables 1 and 9 reveal a conspicuous absence of any constant relationship which might have been expected between control and soil acidity. Failure to bring about satisfactory indications of control is not invariably coupled with failure to bring about a significant increase in soil acidity; and, conversely, plots in which no significant acidification has taken place have, in one experiment (Table 7), shown satisfactory evidences of control. These apparent inconsistencies are interesting inasmuch as they suggest strongly that the problem of control is not a simple one, bound up only with the effect of sulphur upon the soil acidity, but that these two effects may, under appropriate conditions, be quite independent.

In most of the experiments, sulphur dressings resulted in measurable increases of acidity, which, however, vary in magnitude from the merest indication of change (Table 7) to very marked depressions of the pH resulting in acidities considerably greater than that indicated as the limiting acidity for *Actinomyces* by other workers (Tables 3 and 4). In one experiment (Table 9) no sensible change whatever seems to have been effected. Obviously, very small or irregular changes, and those which do not result in acidities approximating the limiting value referred to, cannot be regarded as significant, in this sense, that either by comparison with the others they

are wholly incommensurate with the quantities of sulphur applied, or they do not result in a pH sufficiently low to lead to any expectation of control on that ground alone. For present purposes, we may regard any pH lower than 5.5 as approximating the limiting value for *Actinomyces*. On this hypothesis, the experiments at Guelph, Devlin, and Rosthern (Tables 6, 7, and 9) are the only ones characterized by insignificant acidity changes.

Of these three soils, sulphur had the least effect at Rosthern, while at Devlin and Guelph the acidification was definite, although the lowest pH obtained was not significant for control. In order to see whether excessive alkali reserves were responsible for the behavior of these soils toward sulphur, carbonate determinations were made by the Parr method. The results expressed as percentages of calcium carbonate are given in table 11. It is

TABLE 11.—*The relation of carbonate content of the soil to slight increases in soil acidity produced by applications of sulphur*

Location of experiment	Depression of pH	Percentage CaCO ₃
Rosthern	6.0 to 5.8	0.205
Devlin	7.4 to 6.7	0.337
Guelph	7.1 to 6.2	3.370

clear that at Guelph the carbonate content of the soil may be the cause of the small change in acidity. From the initial acidity of the soil this was hardly to have been expected at Rosthern. But at Devlin, where some success in control was attained, the smallness of the change in acidity is not accounted for by the carbonate content of the soil.

Now, having regard only to the relationship between acidity and control, there are five ways in which any one of the experiments could have resulted: any given experiment might have been disqualified for the purposes of discussion by the absence of a virulent strain of the organism, or it might have resulted in any one of the four possible combinations of presence or absence of satisfactory control with presence or absence of significant acidity increase. Table 10 demonstrates the fact that all of these possibilities were realized in this series of experiments and argues strongly for the independence of the two effects of sulphur under these conditions.

No obvious differences were noted between the two forms of sulphur in respect of their effect either in control or in acidification of the soil. At Plaster Rock (Table 4) inoculated sulphur gave a slightly better acidification than flowers of sulphur, but this situation is reversed at Ottawa and Winnipeg (Tables 5 and 8). At Devlin (Table 7) plots treated with flowers of sulphur appear to have yielded slightly higher percentages of clean tubers than plots treated with inoculated sulphur. All these differences are incon-

siderable, however, and the conclusion indicated would seem to be that neither form of sulphur had any constant advantage over the other.

SUMMARY

1. In these experiments sulphur was not uniformly effective in controlling common scab under all conditions of soil and climate, but gave encouraging results only upon clay soils infested by moderate numbers or by attenuated strains of the organism and under conditions of light rainfall.
2. Evidence is offered of the independence of the effect of sulphur upon soil acidity and its effect upon the disease.
3. No significant difference was observed between inoculated sulphur and flowers of sulphur in respect either of control or of soil acidification.

It would be impossible for the authors to name individually all those to whom they are obligated for the help that made these experiments possible, but especial mention must be made of Mr. H. T. Güssow, Dominion Botanist; Professor J. E. Howitt, Ontario Agricultural College; and Dr. G. R. Bisby, Manitoba Agricultural College. To these gentlemen we are indebted for field facilities, supervision of field operations and many other courtesies. Mr. S. Waterman, of the Ontario Agricultural College, very kindly made the carbonate analyses. To Professor J. H. Faull we owe our thanks for his keen and stimulating interest in the work at all times, and for valuable suggestions and assistance.

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A METHOD FOR TESTING IN VITRO THE TOXICITY OF DUST FUNGICIDES TO FUNGOUS SPORES

H. ATHERTON LEE AND J. P. MARTIN

INTRODUCTION

Eye spot is a leaf disease of sugar cane caused by the fungus *Helminthosporium (Cercospora) sacchari* Butler. Upon very susceptible cane varieties and under favorable conditions for the development of the disease, lesions frequently become so numerous in the young growing spindle that the entire top dies, thus producing a top rot of individual stalks. Material losses are therefore caused by eye spot in small areas in localities where climatic conditions are favorable to the disease.

The application of liquid fungicidal sprays to sugar cane in the usual large fields of most countries is not economically feasible because of the matted and impassable growth of the cane. Under these conditions, however, dust fungicides were found to be readily and cheaply applicable both in field and plot tests in the Hawaiian Islands (4). In field plot tests, with adequate replications, plots treated with Bordeaux dust showed no decrease of eye spot over the control plots. Plots dusted with sulphur showed a slight improvement over untreated plots but the treatment was far from being completely effective. Attempts have therefore been undertaken to secure more effective dust fungicides against the eye-spot fungus, and the following laboratory tests have been developed.

In looking through the literature for similar methods, a procedure suggested by Reddick and Wallace (5) has been noted: it consists of spraying microscope slides with the fungicide to be tested and subsequently placing spores of the fungus on the slide and comparing germination with that of spores on untreated slides. It would seem as if this method was the more feasible for organisms which are cultured with difficulty, but that the method outlined in the present paper is less tedious and more accurate for fungi which are cultured easily. The tests are quicker and also cheaper than field tests. We have used these tests, however, as a preliminary step in selecting new fungicides for subsequent field tests. As we regard them, these tests are an adjunct to field tests of fungicides and should not be considered as a method to supplant field tests.

DESCRIPTION OF THE METHOD

The original plan of the test was taken from the method of determining the phenol coefficients of disinfectants originated by Anderson and McClint-

tic (1). This method was modified slightly by Lee (3) to determine the effect of disinfectants against bacterial plant pathogens. With considerable modifications, it is now being used to determine the effect of dust fungicides against fungi.

Source of Fungous Material to be Tested. The fungus, which is the eye-spot fungus in this case, is cultured in petri dishes on standard nutrient agar plus 2 per cent glucose, pH 7.0, prepared according to the Committee on Bacteriological Technic (2). Cultures are usually maintained at 25° C. in total darkness for nine or ten days, when dense masses of spores are available. It is essential to have the cultures used in the test grown under standardized conditions as nearly as possible.

Apparatus Used. Small round cover glasses, 15 mm. in diameter, are placed in rows on the bottom of glass moisture chambers about 20 centimeters in diameter; these we call, for convenience, dusting chambers. We usually have 50 or 60 such cover glasses to each dusting chamber. The dusting chambers with the enclosed cover glasses are sterilized with moist heat in an autoclave. A suspension of spores from the standardized petri dish cultures of the eye-spot fungus is then prepared in sterile water. Drops of this suspension are examined under the microscope so as to secure 15 to 20 of the fungus spores in a drop. With a sterile 3-mm. platinum loop, one drop of this spore suspension is placed on each of the round cover glasses in the sterilized dusting chambers. All work is carried on in the transfer room and all precautions are taken to avoid contamination of the cover glasses and moisture chambers.

Procedure. The cover of one of the dusting chambers is lifted sufficiently to allow a small insufflator or puff gun¹ to be operated, and a light application of the fungicidal dust to be tested is applied to the 50 or 60 cover glasses having the drops of the spore suspension. We recently have been weighing 2 grams of the dust being tested into the puff gun and expending the total amount in one dusting chamber, so that the amounts of each dust tested are identical. The glass cover of the dusting chamber is then replaced and, following the dusting at time intervals of $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, 6, 10, 12, 24, and 48 hours, five of the dusted cover glasses are removed with sterilized forceps and other aseptic precautions. Each cover glass,

¹ Since this paper was written, Dr. C. R. Orton, at the Boyce Thompson Institute, has shown the writers a device which is superior and more accurate than the puff gun. It consists of an ordinary culture tube, corked, with a blow tube extending through the cork to the bottom of the culture tube. A short outlet tube extends through the cork. When one places the mouth or an ordinary rubber pressure bulb at the end of the blow tube and forces air into the culture tube, the fungicidal dust at the bottom is expelled through the outlet tube and can be directed easily into the dusting chamber. This apparatus is much more easily cleaned and is more accurate than the puff gun.

when it is removed from the dusting chamber, is immediately placed in a culture tube containing 10 cc. of sterile nutrient bouillon.

If the fungous spores are unaffected by the fungicidal dust, growth will occur in the bouillon tubes within two to four days; whereas, if the dust has been toxic to the fungous spores, no growth will result. Working with one fungus such as *Helminthosporium sacchari*, with experience one can instantly recognize contaminations in the bouillon culture tubes. With adequate precautions contaminations are not common.

One cannot be sure that the suspension on the cover glasses contains spores only and no mycelium, but since the mycelium is more quickly affected by the dust than the spores the time interval necessary to prevent growth in the bouillon tubes represents the toxicity to the spores rather than to the mycelium. More recently also our method of obtaining the spore suspension has been to pour the water from a 10 cc. sterile water blank into a 10-day-old culture of the fungus. The spores are then floated off and the suspension pipetted back into a sterile culture tube having a minimum of mycelium as compared to the quantity of spores.

The time intervals for exposure of the fungous spores to the dust may be varied in different tests, according to the discretion of the investigator.

One undusted chamber of 60 inoculated cover glasses is maintained as a control; and whenever dusted cover glasses are placed in the bouillon tubes, five undusted cover glasses are removed to five bouillon tubes as controls. In our laboratory we have usually run tests of four or five different dusts at the same time, with a separate chamber for the undusted controls.

Results. To illustrate the nature of the tests and the results, a few representative trials are presented in tables 1 and 2.

TABLE 1.—*The fungicidal action of various dust preparations on spores of Helminthosporium sacchari*

Treatment, Oct. 2, 1924	Time of exposure in hours						
	1	4	10	12	24	30	46
	No. of bouillon tubes ^a in which growth of the fungus occurred, Oct. 11, 1924						
Control	5	5	5	5	5	5	5
Calcium hydrate.....	5	5	5	4	4	3	1
Du Pont No. 1.....	5	5	5	4	4	2	3
Du Pont No. 12.....	0	0	0	0	0	0	0
Du Pont No. 13.....	5	5	5	3	0	0	0
Du Pont No. 18.....	3	2	0	0	0	0	0

^a In each test five inoculated cover glasses were exposed and transferred to five tubes of sterile bouillon.

The results in table 1 indicate that spores of the eye-spot fungus were killed in one hour by Du Pont dust no. 12, in ten hours by Du Pont dust no. 18, and in twenty-four hours by Du Pont dust no. 13. Du Pont dust no. 1 did not kill the spores readily in forty-six hours; and calcium hydrate, although minimizing germination in forty-six hours, did not inhibit spore germination completely in this test.

Confirmatory results with the same fungicides are shown in table 2.

TABLE 2.—*The fungicidal action of various dust preparations on spores of Helminthosporium sacchari*

Treatment, Oct. 29, 1924	Time of exposure in hours					
	¼	½	1	2	4	7
	No. of bouillon tubes ^a in which growth of the fungus occurred, November 3, 1924					
Controls	5	5	5	5	5	5
Du Pont no. 1	5	5	5	5	5	5
Du Pont no. 12 ...	0	0	0	0	0	0
Du Pont no. 13 ...	2	2	2	1	0	1
Du Pont no. 18 ^b ...	3	1	0	0	0	0

^a In each test five inoculated cover glasses were exposed and transferred to five tubes of sterile bouillon.

^b Although Du Pont dusts No. 12 and 18 have been very toxic to the eye-spot spores, their use on a commercial scale has not yet been tried in field plot experiments.

DISCUSSION

Since the tests tabulated above were made, we have tried out numerous fungicidal dust preparations by these methods. In those cases where we have followed up such tests *in vitro* with field plot tests, the field results have corroborated the laboratory tests with one exception.² *In vitro* the drop of spore suspension usually dries up in from six to twelve hours; and it may be argued that, with the spores dry and none of the fungicide in solution, there will be no action of the fungicide. However, almost identical conditions exist in the fields where eye spot occurs. Under field conditions, the dust is applied to the cane in the early morning while moisture is still on the leaves; in from four to six hours the dew has disappeared and the leaves are dry.

This method indicates the toxicity of the fungicides only in the absence of organic matter, but the results are, of course, comparative.

² Sulphur *in vitro* did not affect spores of the eye-spot fungus, but in field plots treated with sulphur dust there was a slight reduction of eye-spot as compared with untreated plots.

It may be possible to present the results of further tests in a subsequent paper.

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RINGSPOT OF TOBACCO; AN INFECTIOUS DISEASE OF UNKNOWN CAUSE¹

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INTRODUCTION

Our knowledge of the ringspot disease of tobacco dates from 1917, when we observed it for the first time in fields at South Boston, Virginia. The disease was well-known locally at that time and was commonly called ring worm, in the belief that it was caused by insects. We were engaged then and for several succeeding years in a study of blackfire of tobacco, and only occasional notice was given to ringspot. Recently we have devoted more study to this disease and have demonstrated it to be of an infectious nature. Dr. W. D. Valleau informs us that he has obtained similar proof, and at his suggestion we are publishing some phases of our studies at this time.

The disease was first described and illustrated under the name of ringspot by Fromme and Wingard (1) in 1922, and it was discussed in some

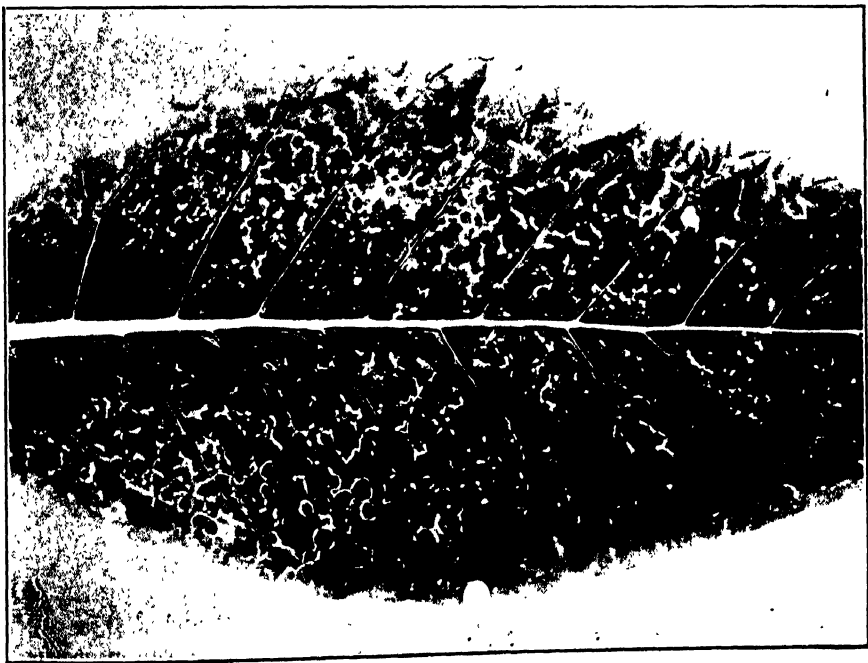


FIG. 1.—Leaf of burley tobacco affected with ringspot. Many of the rings are broken. Natural infection. Appreciably reduced.

¹ Paper No. 69 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

further detail by Wingard and Godkin (7) in 1924. It was considered a non-parasitic disease at this time. Johnson (2) also published figures of a leaf-spot similar to ringspot which he considered non-parasitic, and a figure which was published by Selby (4, plate 1, p. 89) in 1904 strongly suggests the ringspot disease, although it is labeled tobacco mosaic. There is no description in the text of Selby's bulletin which would indicate that a distinct disease of the ringspot type had been recognized. The occurrence of ringspot (also called heiroglyphics) in Virginia, Kentucky and Ohio in 1922 was reported by the Plant Disease Survey in 1923 (5, p. 142), and a further record of occurrence in Kentucky appeared in 1924 (6, p. 295).

OCCURRENCE

At the present time, ringspot occurs quite commonly in the tobacco sections of Virginia, and it has probably been present in the State for a number of years. In a survey of tobacco fields in 1920 it was found in 80 per cent of the fields in Fluvanna County and in 30 per cent of those examined in Charlotte County. It occurred in 1922 in 17 of 28 fields visited in Charlotte County, and we have definite records in this and other years of its presence in Amherst, Bedford, Campbell, Caroline, Halifax, Henry, Pittsylvania, Prince Edward, Russell, and Washington Counties.

The incidence of infection is usually low, and there often are only a few affected plants, in restricted areas of the field. In other fields, however, the incidence of infection has been rather high and severe injury has resulted. An infection of 30 per cent of the plants was noted in several fields in Charlotte County in 1922, and losses in these were appreciable. Some plants have practically all of the leaves affected, while others may show only a spot or two on a single leaf. A severely infected plant may be dwarfed and the leaves may be small, light in weight, and poor in quality. Some plants, however, do not show noticeable dwarfing, and the growth may appear to be normal except for the presence of the spots.

Ringspot is often associated with mosaic in the field but either disease may occur independently. The symptoms are clearly not expressions of the same disease.

SYMPTOMS

The spots occur only on the leaves. They may be uniformly distributed and very numerous, or few and localized. The outline of the spot varies according to the location. They are circular when centered on inter-vein tissue (fig. 6), but when centered on the larger veins they are very irregular in outline. The infection follows the vein and its branches, and the outline of the spot often suggests that of a deeply-lobed leaf (figs. 3 and 4). The spots are bounded by definite broken lines of necrotic tissue which are

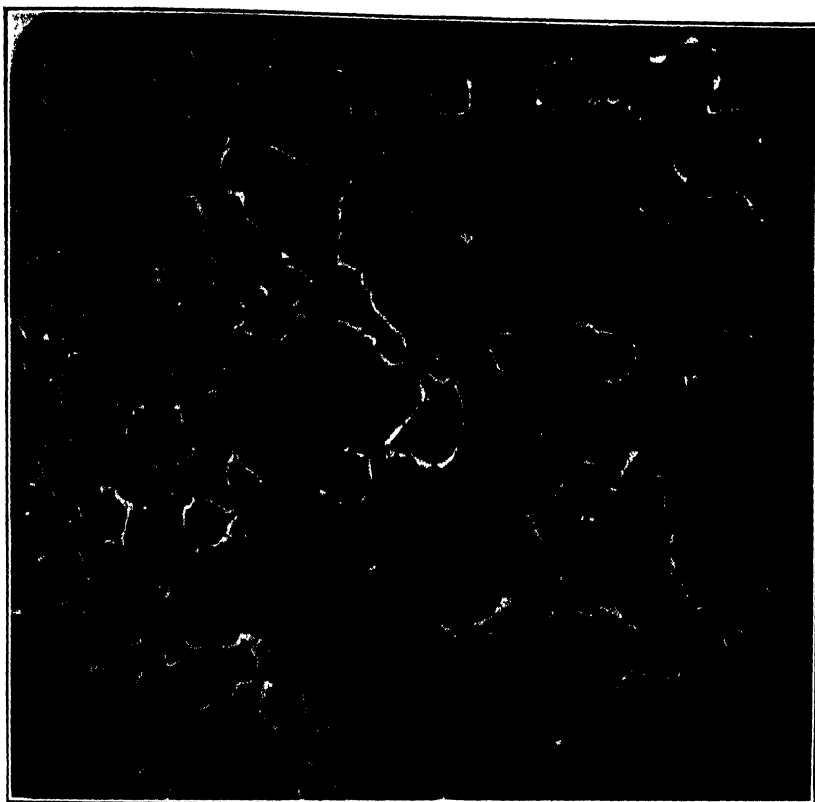


FIG. 2.—Advanced stage of ringspot on a leaf of dark tobacco. Rings are formed on the inter-vein tissue, and spots with irregular leaf-like outlines are formed on the veins. The margins are necrotic and blanching. Natural infection. Slightly enlarged.

blanched or brown in color (figs. 1 and 2). These necrotic margins form rings in the circular spots. Often the rings are doubled (figs. 5 and 6). The rings average about 5 to 8 mm. in diameter, but appreciable variation occurs. The center may be marked by a dot—although in many instances this is lacking—or by a small ring. The margins of the young spots appear as translucent lines in transmitted light, and there may be several zones of these with alternating zones of normal tissue, as in figure 4, A. The leaf tissue which borders the spots may be normal in appearance or it may be chlorotic, and in some varieties it may form a rather definite halo which is suggestive of the halo of the wildfire spot. The appearance of the tissue within the ring varies greatly according to the type of tobacco infected. In some varieties it may be only slightly chlorotic or normal green in color, but in some, which seem hypersensitive, it may become brown and necrotic throughout. The general appearance of the infections suggests the colony

type of growth of an organism or the diffusion of toxic substances from a center.

THE CAUSE OF RINGSPOT

No organism has been obtained consistently in isolations from ringspot and none of those isolated has proved pathogenic. Inoculum of crushed infected leaves, however, has produced infection repeatedly, and subsequent transfer has likewise produced infection. Four successive transfers were made in one instance. Infection is obtained by rubbing the crushed leaf on the trial leaf or by swabbing with a water infusion of affected leaves which have been ground in a mortar. Typical symptoms have been produced in both field and greenhouse. The discussion which follows relates to greenhouse work only.

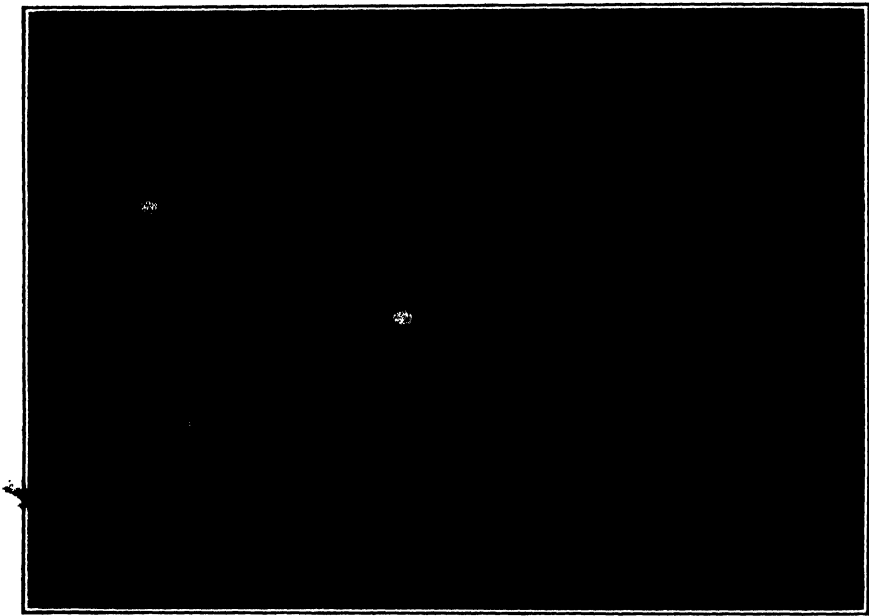


FIG. 3.—Ringspots with irregular leaf-like outlines which arise from vein infection. Natural infection. About twice natural size.

With plants of known susceptibility and with good inoculum one may secure infection in a high percentage of trials. Frequently all of the inoculated plants have become infected.

The incubation period has varied appreciably in different trials but these have covered a wide range of environmental conditions and the test material has been varied. In some cases we have noted the appearance of early symptoms four days after inoculation, and in others twelve or fourteen days have elapsed before definite symptoms appeared. The spots develop best

on young leaves or leaves of intermediate age, and little or no evidence of infection is obtained on leaves which have reached maturity. Young seedlings have been infected, and in one instance we have seen infection in the plant bed.

No extended study of the susceptibility of species and varieties has been made. Plants which were available of a number of species of *Nicotiana* and varieties of *N. tabacum* were tested on a limited scale. Only two or three plants of each type were tested as a rule, and failure to infect was not considered proof of resistance. Infection has been secured on five species of *Nicotiana*, i.e., *glutinosa*, *langsдорffii*, *paniculata*, *sylvestris*, and *tabacum*. The following varieties of *N. tabacum* were infected: *atropurpurea*, *auriculata*, *brasiliensis*, *calyciflora*, *colossea*, *gigantea*, *lacerata*, *latissima*, *macrophylla* and *microphylla*. The agronomic varieties, burley, Green's wildfire resistant, little Orinoco, Macedonian, and Maryland also proved susceptible.

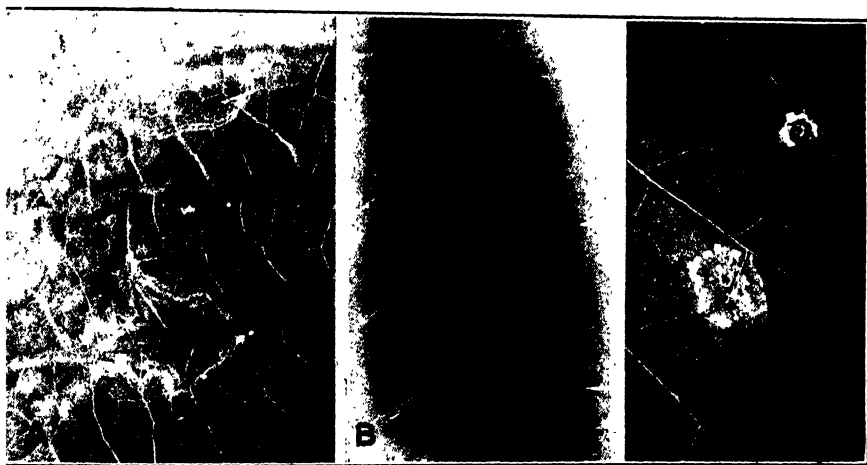


FIG. 4.—A. Ringspots in an early stage of development with margins of alternating zones of chlorotic and normal tissue. The extension of the spots in conformity with the vein pattern is well shown. Natural infection. Transmitted light. About 2 \times . B. In some varieties the margins of the spots appear as dark lines in transmitted light. Natural infection. Slightly reduced. C. Rings which developed from inoculation, as seen in reflected light. Photographed about 30 days after inoculation. Natural size.

A localized, rather than a systemic, infection is suggested by the appearance and occurrence of the spots since they are restricted to the parts of the leaf where the inoculum is applied. Other parts of the leaf remain normal in appearance throughout the life of the plant. There is evidence, however, that infection may become systemic in newly developed parts, especially in the axillary shoots. Ringspots have developed on new, uninoculated leaves of the main stem and suckers, and sometimes all leaves produced above the

point of inoculation have been infected. In one instance inoculum was injected with a syringe into the stem near the ground. The injection was made on October 7, and by November 16 a single ring appeared on one of the upper leaves. On November 30 two newly developed leaves of the top sucker showed infection. In another instance, infection was obtained with the crushed stem of a sucker from which the leaves had been stripped. Further evidence of systemic infection is shown in the behavior of a burley plant which was transplanted from the field to the greenhouse in late summer. With the death of the old infected leaves and the development of



FIG. 5.—Abundant ringspot infection of *Nicotiana tabacum* var. *lacerata* produced with inoculum from a variety of burley. The leaf also bears a few “blisters” of mosaic. Natural size.

new ones the symptoms disappeared, except for a faint mottling, but the leaf extract still proved infectious; and when the stalk was harvested and suckers appeared from the base, these also proved infectious.

As noted previously, ringspot and mosaic are frequently associated in the field and the two diseases evidently have similar potentialities. The incidence of mosaic is usually higher than that of ringspot; it seems to be more infectious or more readily transferred under field conditions. Ringspot is often restricted to a few plants, while mosaic, with an early start, may readily spread throughout the field by harvest. Similar differences in be-

havior are seen in the greenhouse. At one time, mosaic became established in one of the houses and spread so rapidly as to threaten the ringspot work. There has been little or no adventitious spread of ringspot; infection has appeared only on plants which have been inoculated. The same plant may bear both diseases, and inoculum from such plants usually produced both of them (fig. 5).

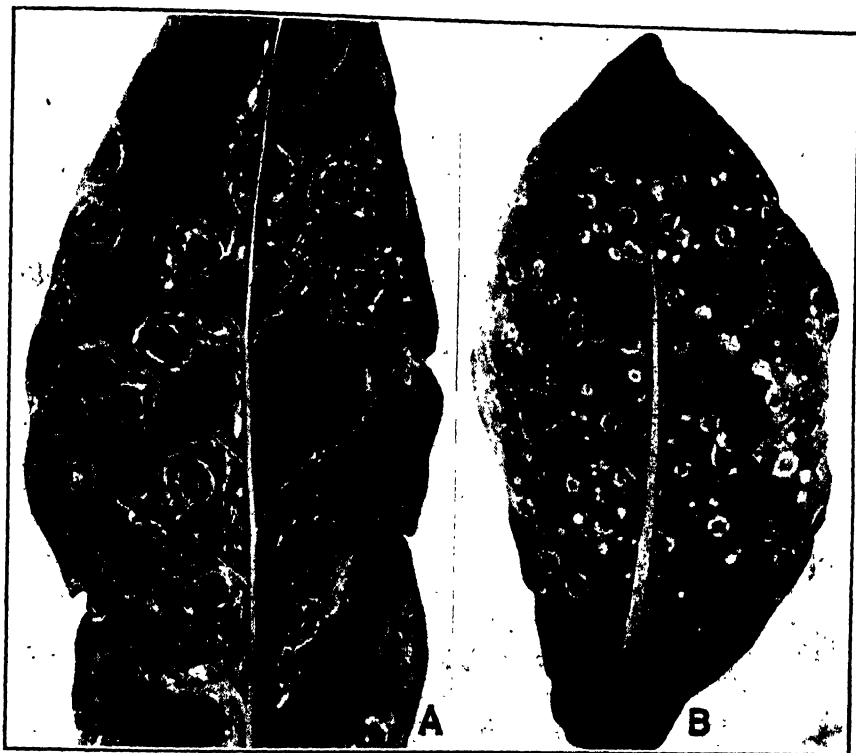


FIG. 6.—Ringspots which developed from inoculation. A. A leaf of a burley variety. Many of the rings are double. Small center rings or dots are seen in some. B. A sucker leaf of *Nicotiana tabacum* var. *microphylla* which developed some time after the inoculation of leaves of the main stem.

Such knowledge as we have would indicate that ringspot may be classed with the virus group of diseases. Its properties seem similar to those of the mosaics of tobacco and other plants, but it is also true that much remains to be learned. Our filtration studies have been too limited to warrant conclusions and we know but little of the factors which inhibit or enhance virulence.

It should be noted that Johnson (3) has produced a type of "ring-spot" from inoculations with juice of apparently healthy potatoes, and he sug-

gests that this disease may prove identical with ringspot of tobacco as it occurs in nature. His figure B, of plate 2, shows definite rings, but their identity with those of the disease which we have described seems questionable. Our inoculations of tobacco with juice of potato leaves have produced no symptoms of ringspot.

SUMMARY

The ringspot disease of tobacco is characterized by the occurrence on leaves of circular or very irregular lesions which are delimited by lines of necrotic tissue. The symptoms vary appreciably in different varieties.

The disease is infectious. It has been produced repeatedly with inoculum of the expressed juice of affected leaves. Transfer of infection is accomplished most readily when the leaves of trial plants are swabbed with the inoculum.

The symptoms may appear after an interval of four days, or longer, and they are at first restricted to inoculated parts of the leaves. The infection may become systemic later in newly developed shoots and leaves.

The disease has been produced in five species of *Nicotiana* and in a number of varieties of *N. tabacum*.

Attempts to culture a pathogen from diseased plants have been unsuccessful. This fact, together with other features of the disease, suggests that it should be classed with the virus group of diseases.

Ringspot occurs rather commonly in Virginia tobacco fields, but it is usually restricted to a low percentage of plants. High incidence of infection occurs rarely, and in such cases losses of consequence may follow.

Natural agencies or modes of transfer are unknown. The disease does not spread under greenhouse conditions, and, in our experience, there has been no adventitious occurrence of infection in the greenhouse.

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PRELIMINARY STUDIES ON WITCHES' BROOM OF STRAWBERRY

S. M. ZELLER

INTRODUCTION

Early in the summer of 1925, Professor M. B. McKay, of the Oregon Experiment Station, discovered in Marion County, Oregon, an unusual disease of strawberry. At that time it was thought probable that this might be the same as the strawberry "yellows" which is so destructive to several strawberry varieties in the Central California Coast District and which has been recently described by Plakidas.¹ Further field study by the writer and a



FIG. 1.—Plant of the Marshall variety of strawberry affected with witches' broom. The larger healthy leaf at the upper left is a hold-over leaf, indicating that infection took place late the previous season. The baby plants at the right have the same symptoms.

This plant was dug and photographed in April.

¹ Plakidas, A. G. Strawberry "yellows," a degeneration disease of the strawberry. *Phytopath.* 16: 423-426. 1926.

comparison of symptoms of the two diseases by Plakidas has convinced us that the two sets of symptoms represent distinct diseases. The spindly habit of growth and multiplication of leaves has led the writer to call the new disease "witches' broom of strawberry."

The general performance of the disease and the suggestion by some growers that, although there is a tendency for it to spread, witches' broom can be kept to a very low percentage by means of roguing, have led us from the first tentatively to place the disease among the degeneration or virus diseases.

OCCURRENCE AND DISTRIBUTION

So far as the writer is aware this disease of strawberry has never been mentioned in literature.

Observations of witches' broom by members of the staff of the Oregon Experiment Station have been confined to the Willamette and Hood River Valleys of Oregon. It seemingly is of rare occurrence in this State. Several growers who produce plants for sale have been interested in the disease for a number of years. Its behavior taught them that baby or runner plants of diseased mother plants were always affected and the natural thing to do was to destroy such plants entirely. This fact and the fact that affected plants are worthless have led to roguing as a natural means of control and have consequently limited the distribution of affected plants. The several varieties which have been observed to be affected with witches' broom are Marshall, Nick Ohmer, Oregon, and Ettersburg No. 121. A strain of Marshall which goes under the names Improved Oregon and Improved Clarke has also been found with symptoms of this disease.

SYMPTOMS OF WITCHES' BROOM

The symptoms of the witches' broom of strawberry are expressed differently on different varieties of strawberry. These may be divided into two main types, those expressed by the diseased Marshall strawberry and those expressed by diseased plants of Ettersburg No. 121. Figure 1 illustrates the symptoms of the disease in the Marshall, although the photograph has not brought out the spindly character of the petioles. In this variety diseased plants have long, usually erect, stiff, spindly petioles upon which are borne leaflets which are much smaller than those of healthy plants. The leaves are usually light green, with a tendency toward olive green shades rather than bright green. The midveins of the leaflets arch downward. The lateral pinnate veins do not show this tendency so much but still enough so that the complete circle of three leaflets has the appearance of curving downward along the entire margin, in direct contrast to the upward cupping of the leaflets in strawberry yellows. There is a tendency for the stems of the

individual leaflets to be longer and for the midvein to be broader and lighter in color than in healthy plants. There is a noticeable turgidity of the leaflets and a crinkliness which is at least uncommon in healthy plants. The great number of leaves with long petioles gives the bushy appearance which suggested the name witches' broom. In the case of Marshall plants, flower stalks are very scarce and, when they do appear, are spindly and unfruitful; while the spindly flower stalks in Ettersburg No. 121 are very numerous and unfruitful.



FIG. 2.—Marshall strawberry plant affected with witches' broom. Many of the leaves have been cut away to show the spindly character of the petioles and the small leaves.

The runners are very much shortened and the baby plants thus take root nearer the parent plants than is normal. The baby plants show the same symptoms as those described for the parent, but the former may show more advanced symptoms than the parent in cases where infection occurred late in the growing season. The root systems of diseased plants appear normal and well developed. Figure 2 illustrates the spindly character of the petioles of a Marshall plant, from which many of the leaves have been cut in order to show the individual leaves.

In the variety Ettersburg No. 121 the brooming or bushy character of the disease is much more pronounced than in other varieties, as is shown in figure 3. This variety naturally grows more bushy than other common varieties; thus this exaggeration of the disease symptoms is not surprising.

In affected Ettersburg plants the internodes of the runners are so extremely short that the baby plants take root right beside the parent crown. Figure 4 shows the parent plant with several baby plants rooted around it, two of them having been pulled aside to straighten and show the runner. The fact that baby plants with their numerous leaves are so closely crowded to the parent is perhaps one reason for the extreme brooming in diseased specimens of this variety. The leaves of the diseased Ettersburgs are not arched downward at the margins so decidedly as in the other varieties.

Plants which are too deeply planted sometimes make a peculiar growth which might be mistaken for symptoms of witches' broom. When baby plants are completely covered in cultivation, a spindly type of plant may result. To observe closely, however, in the case of the Marshall variety, the texture of the leaves of plants recovering from such unnaturally deep submergence in soil can be seen to be extremely delicate and pliable, in contrast to that of leaves of plants diseased by the witches' broom virus. The mar-

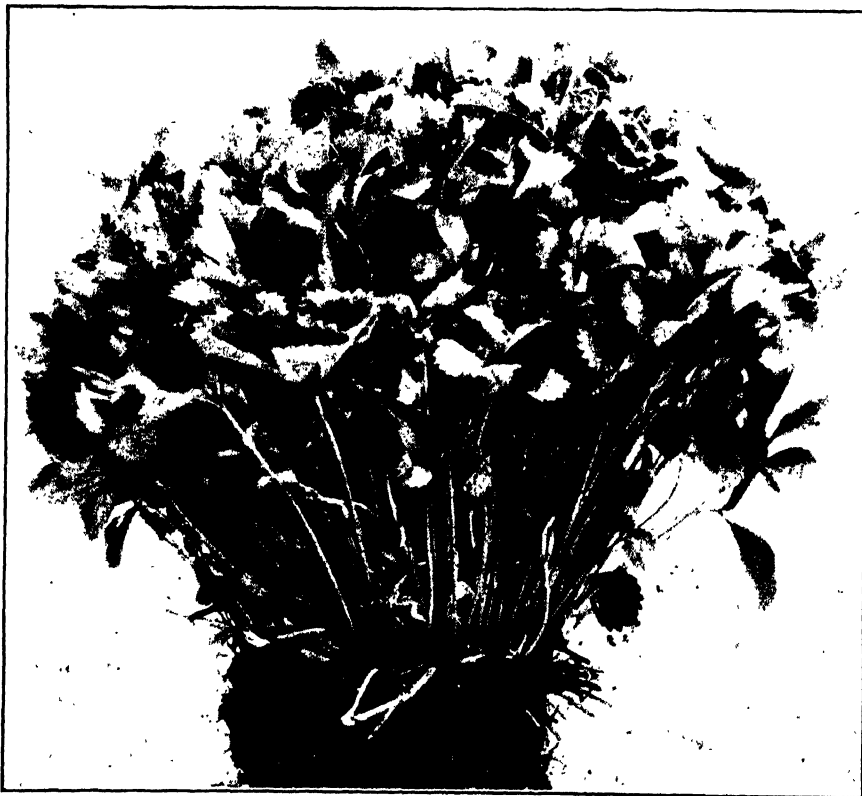


FIG. 3.—Plant of the Ettersburg No. 121 strawberry affected with witches' broom. This variety shows the "brooming" effect more than other varieties.

gins of the leaves of these deeply-planted specimen are definitely crenated but not particularly downward curved.

NATURE OF WITCHES' BROOM

Preliminary experimental work has led to the conclusion that witches' broom of strawberry is a virus disease which may be transmitted by insects. Transmission experiments were conducted during the season of 1926. Twenty-five healthy plants of the Marshall variety were enclosed in insect-proof cages, one plant in each cage. In addition, several diseased plants of Marshall and Ettersburg No. 121 were caged individually. For a transmission agency the strawberry leaf louse (*Myzus fragae-folii*) has been used.

The insect-proof cages for individual strawberry plants were constructed in the following manner. Half-inch-mesh chicken wire 36 inches wide was cut into strips 36 x 12 inches. Each strip was bent into a cylinder 12 inches high and approximately 12 inches in diameter so that the 12-inch edges could be stapled to a stake, 18 x 1½ x 1 inch, leaving the upper end of the stake flush with the wire mesh and 6 inches projecting below. The stake was inside the cylinder.



FIG. 4.—An Ettersburg No. 121 plant in which the baby plants have been pulled away from the parent to show the shortened internodes of the runners.

A very good grade of cotton sheeting (L L Caddo) was cut into 20-inch lengths and the selvage edges sewed together to form tubes. A cloth tube so made may be slipped snugly over the cylindrical wire frame until its lower edge can be hooked over the cut ends of the wires below. The 8 inches remaining above may be tied with a string. The cage is set over the plant and the 6-inch stake driven into the soil to hold the cage in place. A little soil placed around the base of the cage makes that portion satisfactorily insect-proof. An opening of any desirable size for making observations may be made where the upper end of the cloth tube is tied.

Aphis (leaf lice) obtained from healthy plants growing in a planting of Marshalls where no diseased plants were found were placed on some caged, diseased plants and on four of the caged healthy plants. After eight days, leaves harboring from two to three aphis were transferred from plants in these cages to healthy Marshall plants in insect-proof cages. From two to three aphis from diseased plants of Ettersburg No. 121 obtained from a commercial planting were transferred to each of several Marshall plants in cages. After about three to four weeks the new leaves appearing on some of the healthy Marshall plants to which aphis had been transferred from diseased Marshall or Ettersburg plants showed symptoms like those illustrated in figure 1. The results of the transmission experiments were as follows. Aphis transferred from the four healthy Marshall plants to as many healthy Marshall plants proved to be nonviruliferous, the plants remaining healthy six months after the insects were transferred to them. Nine out of fourteen Marshall plants have shown the symptoms of witches' broom in three to four weeks after aphis which had fed on diseased Marshall plants for 8 days were transferred to them, and 5 out of 7 Marshall plants showed the same symptoms within the same period after aphis which had fed for 8 days on diseased Ettersburg plants were transferred to them. After six months the same condition held, the symptoms having gradually become more and more apparent.

Although there was not a high percentage of transmission in either case, there is conclusive evidence that the disease is caused by a virus which may be transmitted by the aphid, *Myzus fragaefolii*. It is also apparent that the two groups of symptoms, those shown by the Marshall variety (Fig. 1) and those shown by the Ettersburg variety No. 121 (Fig. 3), are both expressions of the same virus disease, witches' broom. Transmission of the disease has not been attempted with any other insect to any extent. *Myzus fragaefolii* is therefore at present the only known agent or means by which the virus of witches' broom of strawberry can be transmitted. The disease has not been studied in its relation to climatic or other ecological factors.

SUMMARY

Witches' broom of strawberry is characterized by a dwarfing of the whole plant, spindliness of petioles and an arching downward of the margins of the leaflets, which are lighter in color than in normal plants. Runners are shortened, the baby plants with symptoms like those of the mother plant taking root near the parent. More broominess is exhibited by some varieties, like Ettersburg No. 121, than by other varieties.

Witches' broom has been found in western Oregon only, but may have wider distribution. Varieties have not been tested for resistance or susceptibility, but Marshall, Nick Ohmer, Oregon, and Ettersburg varieties have been found affected. Viruliferous leaf lice (*Myzus fragaefolii*) transmit the disease.

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PHYTOPATHOLOGICAL NOTES

A Wilt Disease of Alfalfa Caused by Fusarium sp. In September, 1926, H. L. Westover found a disease attacking alfalfa plants, about six months old, growing in his plots at the Government Experiment Station at West Point, Mississippi. The disease appeared similar to, but not quite typical of, the bacterial wilt caused by *Aplanobacter insidiosum* L. McC., recently described by Jones¹ and Jones and McCulloch.² Therefore, specimens were submitted to the writer for study. Westover stated in a letter that a large number of plants were dying and that many others had a sickly appearance. The affected plants appeared to be somewhat dwarfed and in some cases were dying from the top downward. The symptoms, however, were not very definite. When the bark was removed from a root or when cross sections of a root were examined macroscopically, a brown or reddish-brown discoloration of the xylem was evident. In early stages of the disease this discoloration appeared to be localized in the young xylem near the cambium. In some cases there was a more or less complete ring of discolored tissue, while in others only one or more bundles were thus affected. In advanced stages the entire woody cylinder was discolored. The discoloration could often be traced from the root into one or more shoots for a distance of three or four inches.

The writer has not had an opportunity to study the symptoms of this disease in the field, but the following points will help to distinguish it from the bacterial wilt, the disease with which it may possibly be confused. Although the color of the tissues affected with bacterial wilt varies, it is usually a shade of yellow or light-brown as contrasted with the darker brown or reddish-brown of those affected with *Fusarium* wilt. When viewed microscopically in cross section, the coloring matter in roots affected with bacterial wilt appears to be located largely within the lumina of the vessels, while in roots affected with *Fusarium* wilt the color is in the cell walls.

Isolations made by planting some of the discolored tissue on agar yielded a species of *Fusarium* in almost every instance. Plants of different ages growing in the greenhouse were inoculated with this *Fusarium* by four different methods. Infection resulted in a large percentage of the tests, and the fungus was reisolated from nearly every infected plant. The details of these experiments, together with others now under way, will be given in another paper. Under greenhouse conditions the disease first becomes evi-

¹ Jones, F. R. A new bacterial disease of alfalfa. *Phytopath.* 15: 243-244. 1925.

² Jones, F. R., and Lucia McCulloch. A bacterial wilt and root rot of alfalfa caused by *Aplanobacter insidiosum* L. McC. *Jour. Agr. Res.* 33: 493-521. 1926.

dent about two months after inoculation by the yellowing of the leaves and a gradual dwarfing and dying of the plants. Affected plants may wilt during the hottest part of the day and become turgid again later. The xylem of such plants always has more or less darkened areas as in naturally affected plants.

Nothing is known yet regarding the distribution of this disease, as it has never been seen except in the original collection. Although a detailed discussion of the pertinent literature will not be given at this time, a brief summary seems desirable. Several writers have reported a root rot, or wilt, or both, of alfalfa with which a species of *Fusarium* has been associated. In several cases the associated fungus is said to be *Fusarium roseum*. The *Fusarium* being studied by the writer is not that species. Most of the reports of *Fusarium* root diseases of alfalfa are so meager that they do not give very clear pictures of the true nature of these troubles. The most comprehensive descriptions of so-called *Fusarium* diseases are those by McCallum³ and Cottam.⁴ However, neither of these writers reproduced the disease experimentally. Arnaud⁵ describes a disease of alfalfa in France as due to *Neocosmospora vasinfecta*.

Further study and field observations are necessary to determine whether the disease herein described is the same as any hitherto reported.

The writer would appreciate it very much if other workers would send him alfalfa plants suspected of having this disease, together with information regarding any damage it may be doing. Further studies are under way with a view to determining the identity of the causal fungus and other facts relative to its spread and control.—J. L. WEIMER, U. S. Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station.

Loose Kernel Smut on Feterita. Infections by *feterita* by *Sphacelotheca cruenta* (Kühn) Potter apparently have not been previously reported. Reed⁶ inoculated seed of *feterita* with spores of this fungus, but no smutted heads resulted. The following observations were made by the writers at the U. S. Field Station, San Antonio, Texas, where seed of *feterita* (C. I. No. 182), which had been sprayed lightly with formaldehyde (one

³ McCallum, W. B. Vegetable physiology and pathology. Ariz. Agr. Exp. Sta. Ann. Rept. 18: 230-232. 1907.

———. Plant physiology and pathology. Ariz. Agr. Exp. Sta. Ann. Rept. 19: 357-361. 1908.

⁴ Cottam, W. P. A "dry rot" disease of alfalfa roots caused by a *Fusarium*. Phytopath. 11: 383. 1921.

⁵ Arnaud, G. Une nouvelle maladie de la luzerne. Prog. Agr. et Vit. (Éd. l'est-Centre) 54: 517-519. 1910.

⁶ Reed, George M. Varietal resistance and susceptibility of sorghums to *Sphacelotheca sorghi* (Link) Clinton and *Sphacelotheca cruenta* (Kühn) Potter. Mycologia 15: 132-143. 1923.

pound to 10 gallons of water), was sown on March 24, 1926, in the sorghum varietal experiments. There was no evidence of smut when the plants were harvested on July 23. Because of rains following harvest, the feterita plants made a second growth and finally produced a second crop of heads. On September 28, when the seed of the second growth was in the dough stage, about 10 per cent of the heads were found to be infected with loose kernel smut. This indicated that the fungus had entered the plants, probably in the seedling stage, but that its development had been retarded so that the fungus did not reach the heads of the first crop. Hence the apparent immunity of feterita from loose smut infection may be merely the result of a retardation of the growth of the fungus within the host. This would be in the nature of escapement rather than immunity. The smut was identified as *Sphacelotheca cruenta* by Dr. W. H. Tisdak —J. H. MARTIN, Office of Cereal Crops and Diseases, and G. T. RATLIFF, Office of Western Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture.

Butt Rot in Diospyros virginiana caused by Polyporus spraguei. For several years a butt rot in living trees of the American persimmon (*Diospyros virginiana* L.) has been observed in southern Indiana. This rot is unusual because so far as the writer knows no heart rot of persimmon has ever been reported and because it was formerly thought that the peculiar black impregnation of the heart wood would probably inhibit the development of wood-destroying fungi. This belief was based on an experiment in which *Polystictus versicolor* failed to develop on a medium made with a decoction from the heart wood.

The butt rot was first observed in old basal scars in trees growing singly in fields. There was never any evidence of the presence of a fruiting fungus, and several examinations of the decayed wood failed to show mycelia that could be attributed to any of the usual wood-destroying fungi. On one of the writer's periodical visits to southern Indiana (Nov. 7, 1918), a large living field tree was found blown down. Growing directly from the decayed heart wood of the splintered butt was a small, poorly developed fructification of *Polyporus spraguei* B and C. The decay, naturally of a darker color in this wood than in any other, was in other characters typical of this fungus *viz.* carbonaceous. From the manner of the development of the fructification directly from the decayed wood, also from the fact that wood-destroying fungi of the first rank rarely find in wood already utilized by another fungus the nourishment necessary for their development, it is assumed that the decay in question was caused by *Polyporus spraguei*.

It is interesting to record that in the wet wood in the base of the stump nematodes were found. Since this time nematodes have been found in decayed wood of various trees and in rotted logs on the ground. Recently Dr.

N. A. Cobb has announced to the writer that he has also found nematodes in rotted wood. In light of the known enzymatic activity of wood-destroying fungi, the role of these organisms can only be a secondary one.

Indications are that *Polyporus spraguei* is a fairly common wound fungus. The writer has called attention to it as causing a butt rot in *Quercus* and *Castanea*.¹ The fungus is usually found on dead wood of *Quercus*, *Castanea*, *Fagus*, and rarely on *Fraxinus*. The fungus apparently prefers the hard, firm woods.

Polyporus spraguei is not supposed to occur in Europe. A form on chestnut in France has been described by Bourdot and Galzin² under the name *Polyporus castaneae*. The type of the species in its gross characters is indistinguishable from the American plant. The minute characters are as follows: Spores ovoid to globose hyaline, uniguttulate apiculate, $5.5-7 \times 4.5-6 \mu$, tramal hyphae homogenous $2-4 \mu$, context hyphae $2-4 \mu$ rarely $5-6 \mu$. The microscopical characters of the type of *Polyporus spraguei* (Sprague 822, 974, Herb. Curtis 5700 and 5718 collected by Dr. Murray in Massachusetts) are--spores ovoid to subglobose hyaline, uniguttulate, apiculate average $5-6.5 \times 4-5.5 \mu$, tramal hyphae homogenous $2-3.1 \mu$, context hyphae $2-4.5 \mu$. The writer collected the European fungus in France in 1910 on *Quercus*. The decay was found to be the same as that caused by the American fungus in *Quercus*. It appears that the two forms should not be held distinct.—JAMES R. WEIR, United States Department of Agriculture, Washington, D. C.

¹ Weir, James R. *Polyporus spraguei* Berk., cause of heart rot. *Phytopath.* 13: 288. 1913.

² Bourdot, H., and A. Galzin. *Hymenomycetes de France II. Pores.* *Bull. Myc. Soc. Fr.* 41: 105. 1925.

BOOK REVIEW

Manual of Plant Diseases. By F. D. Heald. Edition I, 891 pages, 272 figures. 1926. McGraw-Hill Book Co., Inc.

Plant pathologists of America have for years felt the need of a text adequately covering the fundamentals of their field. "Fungous Diseases of Plants" by Duggar, issued in 1909, has for some years been inadequate owing to the rapid advances which have been made in the field of pathology and mycology. Manuals of diseases of special crop groups have been published. They, however, have not satisfied the requirements for a college text presenting the fundamentals of the subject. The teaching pathologist will welcome the appearance of Heald's "Manual of Plant Diseases," which is a book for the class room, based on lectures of the author prepared for his classes.

The book is divided into four sections, the first devoted to an introduction which includes a historical sketch of the science and its development and a second chapter on symptoms of disease in plants.

The second section is devoted to a consideration of non-parasitic diseases. There are nine chapters in this section, covering some 170 pages. The chapters are devoted to such subjects as "Diseases due to—deficiencies in food materials in the soil;—excesses of soluble salts in the soil;—unfavorable water relations;—improper air relations;—high temperatures;—low temperatures;—unfavorable light relations;—manufacturing or industrial processes; and—control practices." This is a very welcome feature as this phase of plant diseases needs to be brought to the attention of our students more than has been the custom in the past. Diseases due to environmental and soil conditions and to parasites dovetail so frequently that the student unfamiliar with the first is blind to an important phase of his work. Developing this phase of the subject before the parasitic diseases is a good arrangement, as it provides a good backing for the latter.

The third section, consisting of one chapter covering 55 pages, is devoted to "Virus and Related Diseases" and embodies a general discussion of diseases of this type with detailed consideration of Peach yellows, Little peach, Potato leaf-roll and Potato mosaic. Other diseases of this type are briefly described or listed with reference to important contributions.

The main, or fourth, section, occupying about two-thirds of the book, takes up "Parasitic Diseases" and consists of 16 chapters. The last two chapters of this section are devoted to the consideration of parasitic seed plants and Nematodes, and the diseases they cause. The major portions of the section are devoted to diseases caused by bacteria and fungi. Some 40 diseases caused by members of these two groups are given major consideration, and are grouped according to the mycological position of the pathogenes. In this section the author gives considerable attention to the morphology of the bacteria and fungi. The figures for this phase of the study are especially clear, and will be found helpful to those studying morphology. The discussion of the diseases is developed around such paragraph headings as History and Distribution, Symptoms and Effects, Etiology, Predisposing Factors, Host Relations, and Control. (One misses a detailed discussion of the vascular wilts.)

The discussions are clear and unusually free from technical language. The photographs are well chosen and the majority well reproduced; a few do not show the detail they should.

In the writer's opinion Doctor Heald has prepared a good and acceptable book. Some of the matter presented, however, might wisely have been condensed to give space for a discussion of the principles of parasitism, resistance and susceptibility, inoculation, infection, dissemination, the relation of environment (soil and air temperature, humidity and soil reaction) to infection and development of infectious diseases, the fundamental principles of control. Much of this is presented in the book in the consideration of specific diseases but not in a connected form which would be of value and help to the student and the instructor.

The mechanical construction of the book is good; the type is clear and large and the book as a whole is that of McGraw-Hill standard.—A. B. MASSEY, Virginia Polytechnic Institute.

REPORT OF THE EIGHTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The eighteenth annual meeting of the Society was held at Philadelphia, December 28-31, 1926, in conjunction with the American Association for the Advancement of Science. The headquarters were at the Hotel Normandie and about 200 members were in attendance.

The program contained 61 papers, 9 in general session, 3 in joint session with Section G, 12 in joint session with the mycological section of the Botanical Society of America, and the remainder grouped in sections under cereal diseases (7), fruit diseases (8), vegetable diseases (15), and fungicides (7). Abstracts of papers were distributed at the meeting and were published in the January number of the 1927 volume of PHYTOPATHOLOGY.

A popular account of the meeting by W. A. McCubbin and H. W. Thurston has been printed in the report of the Permanent Secretary of the American Association (Science 65: 106-107. 1927).

The papers presented at the joint session with Section G were: *Leaf Structure and Wound Response*, by R. B. Wylie; *The Accumulation of Electrolytes*, by W. J. V. Osterhout, and *Vigor of the Host as a Factor in the Development of Disease*, by F. D. Fromme.

A plant disease survey round-table session was introduced for the first time this year. Reports were made on disease conditions in the various states, and specimens and photographs of new or otherwise interesting diseases were exhibited. The meeting was held at the hotel headquarters and was attended by about 80 members.

The conference on extension work in plant pathology devoted its afternoon session to a discussion of the wheat bunt situation, the subject of the copper-carbonate seed treatment being considered in detail.

A very instructive and pleasurable feature of the meeting was the Friday excursion to Wilmington and vicinity as guests of the E. I. du Pont de Nemours & Company. About 100 persons took the trip which was by boat to the company's plant at Deep Water, New Jersey, and to Wilmington. Busses then conveyed the party to the Wilmington Country Club for lunch and later to the wonderful conservatory of Mr. P. S. du Pont at Kennett Square, Pennsylvania. At Deep Water the buildings and equipment of the Jackson laboratories were inspected, and the experimental work, as well as a motion picture on seed and soil disinfectants, were seen.

The phytopathologists' dinner at the Hotel Normandie was attended by 194 persons. The dinner program was especially arranged in honor of Dr. Erwin F. Smith, who has contributed so much to our science during the 40 years of his service in the Department of Agriculture. After appropriate remarks, the president, I. E. Melhus, introduced Dr. L. R. Jones, who spoke on Doctor Smith's service to plant pathology. He was followed by Dr. W. H. Welch, of Johns Hopkins University, who spoke on Doctor Smith's contributions to human and animal pathology. Dr. F. V. Rand, chairman of the committee on arrangements, then presented Doctor Smith, in the name of the Society, with a beautiful leather-covered brochure in which were engrossed the abstracts of speeches that had just been made, followed by the autographs of the members present. Other features of the dinner program were singing led by Donald Porter, a report on an official seal for the Society by H. B. Humphrey, and a report by Donald Reddick, secretary of the plant pathology section of the International Plant Congress at Ithaca.

OFFICERS AND REPRESENTATIVES

The following officers were chosen, the first five being elected by the Society under the new method of balloting, and the others selected by the council and approved by the Society.

President, M. F. Barrus, New York State College of Agriculture, Ithaca, N. Y.

Vice-President, H. P. Barss, Oregon Agricultural College, Corvallis, Ore.

Councilor (two years), C. L. Shear, Bureau of Plant Industry, Washington, D. C.

Representatives on Board of Control of Botanical Abstracts, F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va. (four years); E. C. Stakman, University of Minnesota, St. Paul, Minn. (two years).

Associate Editors of Phytopathology (three years), Freeman Weiss, Bureau of Plant Industry, Washington, D. C., to complete the unexpired term of W. H. Tisdale, resigned; Leslie Coleman, University of Toronto, Toronto, Canada, to succeed B. T. Dickson; M. N. Levine, University of Minnesota, St. Paul, Minn., to succeed A. W. Henry; F. J. Schneiderhan, Virginia Polytechnic Institute, Blacksburg, Va., to succeed C. A. Ludwig.

Business Manager (one year), R. J. Haskell, Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council for the American Association for the Advancement of Science (one year), G. P. Clinton, Agricultural Experiment Station, New Haven, Conn., and Donald Reddick, New York State College of Agriculture, Ithaca, N. Y.

Representative on Committee on American Type Culture Collection (three years), C. L. Shear, Bureau of Plant Industry, Washington, D. C.

Members of the Advisory Board (three years), E. L. Nixon, Pennsylvania State College, State College, Pa., to succeed N. J. Giddings as commissioner for the Northeast; J. G. Dickson, University of Wisconsin, Madison, Wis., to succeed M. W. Gardner as commissioner for the Mid-West.

Member of the Board of Governors of the Crop Protection Institute (three years), H. W. Anderson, University of Illinois, Urbana, Ill., to succeed M. F. Barrus. The other two members of the board are N. J. Giddings and I. E. Melhus.

Representative on the Division of Biology and Agriculture of the National Research Council (one year), F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

The following temporary committees were appointed by the President to serve throughout the meetings: *Resolutions Committee*, L. M. Massey, H. A. Edson, and I. E. Melchers; *Auditing Committee*, G. K. K. Link, E. S. Schultz, and J. B. Kendrick; *Committee on Publicity*, W. A. McCubbin and H. W. Thurston, Jr.; *Committee on Dinner Tickets*, R. S. Kirby and W. A. Archer; *Committee on Meeting Rooms*, I. E. Vogel.

REPORT OF THE SECRETARY-TREASURER, 1926

At the close of the Kansas City meeting the membership totaled 672. During the year 5 were reinstated, and a loss of 27 was sustained—20 by suspension for non-payment of dues, 4 by resignation, and 3 by death, making a total membership of 650 at the close of 1926. Of these, 115 were life members and 535 regular members. At Philadelphia 66 new members were elected, thus bringing the total up to 716. Of the new members 12 came from New York, 6 each from Iowa and Japan, 5 from Wisconsin, 4 each from California and Florida, 3 each from Canada and Minnesota, 2 each from Indiana, Illinois, England and China, and 1 each from 15 scattered states and countries.

STATEMENT OF ACCOUNTS FOR 1926, AS OF DECEMBER 20, 1926

Receipts:

Balance from 1925	\$1,992.41
Annual dues: 1923	4.00
1924	4.00
1925	49.80
1926	987.30
1927	1,594.98
1928	5.00
1929	4.00
1930	4.00
	<hr/>
	2,653.08
Excess dues	6.25
Interest on checking account	47.43
Cash returned by secretary-treasurer on expenses	51.59
Dues in Phys. Section received with annual dues	1.00
	<hr/>
	\$4,751.76

Expenditures:

Member subscriptions transferred to Phytopathology	\$1,777.00
Secretarial work	96.00
Postage stamps and registry	37.17
Telegrams	1.60
Printing (preliminary announcements, dinner tickets, lists of members, letterheads, nomination blanks, ballots, envelopes)	78.63
Expense of motion picture apparatus at Kansas City meeting	17.50
Miscellaneous expenses of Phytopathological dinner	15.00
Check returned by bank	4.00
Expenses of Committee on International Botanical Congress	296.06
Sales received with dues transferred to Phytopathology	7.50
Purchase of stamped envelopes, printed	79.26
Donations to European subscribers transferred to Phytopathology	60.98
Excess dues refunded	1.00
Dues in Phys. Section paid	1.00
Advanced to Secretary-Treasurer for expenses at Kansas City meeting	150.00
	<hr/>
	2,622.70
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Balance	2,129.06
Outstanding check	1.00
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	\$2,130.06
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Amount of above receipts credited to 1927-1930	\$1,607.98
Amount due sinking fund	294.00
Member subscriptions due Phytopathology	31.00
	<hr/>
	1,932.98
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Actual balance for 1926	197.08

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1926

PHYTOPATHOLOGY continues to be in good condition financially even though the bank balance at the close of the year, \$1,103.84, is less than that of last year by \$1,167.55. This is largely due to the publication of a larger volume of Phytopathology this year. On account of the increased number of pages and illustrations, it cost some \$1,400 more to manufacture it in 1926 than it did in 1925.

The number of subscribers in good standing on the list at the close of the year is 419, of which 254 are foreign and 165 domestic. This is an increase of 12 over the number on the list a year ago. The present edition of PHYTOPATHOLOGY is 1,475 copies, of which 1,069 are being mailed out.

Advertising receipts increased from \$1,110.78 in 1925 to about \$1,434 in 1926. Credit is due the Advertising Manager for steady and persistent work in securing new advertisements and retaining old ones.

The sinking fund has now passed the \$5,000 mark, and is \$5,044 to be exact.

STATEMENT OF ACCOUNTS FOR 1926, AS OF DECEMBER 20, 1926

Receipts:

Balance from 1925	\$2,271.39	
Subscriptions (\$424.19 for 1927 and 1928)	1,885.88	
Sales	612.77	
Advertising: 1925	\$ 147.80	
1926	1,273.84	
1927	35.30	
	<hr/>	\$1,456.94
Interest on sinking fund and investment	368.75	
Member subscriptions for 1926	1,777.00	
Donations to European subscribers for 1924 and 1925	60.98	
	<hr/>	8,433.71
		\$8,433.71

Expenditures:

Manufacturing Phytopathology:

Vol. XV, No. 12	\$ 535.39	
Vol. XV, Index	136.14	
	<hr/>	\$ 671.53
Vol. XVI, No. 1	530.44	
Vol. XVI, No. 2	445.89	
Vol. XVI, No. 3	521.02	
Vol. XVI, No. 4	446.52	
Vol. XVI, No. 5	363.28	
Vol. XVI, No. 6	366.41	
Vol. XVI, No. 7	356.03	
Vol. XVI, No. 8	498.79	
Vol. XVI, No. 9	547.04	
Vol. XVI, No. 10	566.65	
Vol. XVI, No. 11	833.37	
Engravings for Vol. XVI	840.33	
	<hr/>	6,315.77
		<hr/>
		6,987.30

	\$6,987.30	
Printing Phytopathological Abstracts	37.90	
Expenses of Editor-in-Chief (letterheads, postal cards)	18.79	
Expenses of Advertising Manager (postage and stenography)	30.35	
Postage, mailing back volumes from College Park	17.76	
Secretarial work	202.50	
Miscellaneous expenses (graphs, photograph)	7.00	
Duplicate subscriptions refunded	13.27	
Subscriptions to other journals sent by error refunded	5.00	
Postage, Business Manager	10.00	
	<hr/>	
	\$7,329.87	\$7,329.87
Balance		<hr/>
Outstanding checks		914.98
		<hr/>
		\$2,018.82

Assets:

Cash in bank (balance shown in statement of account)	\$2,018.82
First mortgage (surplus)	1,000.00
Sinking fund (invested)	4,750.00
Amount due sinking fund (now in Society's account)	462.00
Amount due from subscriptions	467.75
Amount due from advertising	121.30
Amount due from sales made	61.40
Member subscriptions due to date	672.00
Cash and checks on hand to be deposited	164.40
	<hr/>
	\$ 9,717.67
Estimated value of back numbers of PHYTOPATHOLOGY for sale	13,500.00
	<hr/>
Total assets	\$23,217.67

Liabilities:

None.

REPORT OF THE EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

Volume 16 of PHYTOPATHOLOGY contains 1,012 pages, comprising 79 articles, 21 notes, 5 reports and 4 book reviews; 55 plates and 124 text figures. About 100 manuscripts were submitted. Of these, 19 were returned for revision and 11 rejected.

There were two hundred pages more in volume 16 than in volume 15. Fewer manuscripts were handled. On the average, the papers have been longer. The number returned for revision this year, compared with 35 during 1925, indicates that the manuscripts were more carefully prepared than last year. However, perfection has not yet been attained. Authors should consider more carefully methods of presentation, such as subdivision of the article into sections, methods of citing literature, table headings, and legends for illustrations. All authors should have some one with good critical sense read their manuscripts before they are submitted for publication.

The average length of time required to get papers into print, on the basis of the number published during 1926, has been five months from the date of submittal to the

month of issue of the Journal. It is doubtful that this time can be shortened very much. The editor does not always find it possible to read a paper on the same day on which it is received. Manuscripts often must be sent to other members of the Editorial Board and to other people for criticism and advice, then they must be marked for the printer, authors must be consulted by mail, and considerable time is usually consumed in the printery. The sending back and forth of proofs and all the other necessary details usually require at least four months.

The tendency of many authors to ignore previous work along the same line is becoming very pronounced. While it ought not to be necessary to go back to Theophrastus in the literature review of every paper, it certainly borders on the unethical to let an article carry the imputation that it contains the results of first work done along that line. There is, of course, a mass of literature pertaining to many subjects. However, it should be possible to refer to at least some of the more important of the previously published papers. A solution of this problem can not be reached by mathematical formulae. It is respectfully urged, however, that all contributors say the Golden Rule over to themselves several times and then think about it before they begin their literature reviews.

Prolixity is a fault in many manuscripts. Papers should be just as concise as possible: all irrelevant and unimportant matter should be eliminated. Most manuscripts could be reduced 25 per cent without decreasing their value. In fact, the value of many of them would be increased. More good material can be published if every one tells his story in as few words as possible.

Illustrations must be improved. Many of the prints submitted could not possibly show any details after reproduction. Authors should give thought, also, to the arrangement of the figures in plates. They should be neatly and clearly labeled, and the legends should be concise but clear. Illustrations should add something to the value of the article—many of them do not.

While there has been considerable improvement in manuscripts received this year, far greater improvement can and should be made.

REPORT OF THE ADVERTISING MANAGER

The advertising returns have shown a reasonable increase for the year 1926. The total number of advertisements appearing in the 12 issues is 156 or an increase of 24 per cent. This total of advertisements was distributed as follows: 49 full pages; 67 half pages; 34 one-fourth pages; and 6 one-eighth pages, making a total of 91½ pages of advertising or 22½ pages over 1925, representing a 24 per cent increase.

We have experienced better cooperation this past year with commercial companies manufacturing spray materials. However, it has been difficult to develop contact with those concerns manufacturing spray and laboratory equipment. It would materially assist to increase our advertising returns if members would cooperate and assume a little initiative in developing contact through representatives of companies with whom they are acquainted which should be among our list of advertisers. Only three members this past year assisted in securing our advertising. It is our experience that personal appeal and contact only is productive of results. The wide territory that our membership represents should certainly assist in broadening the possibilities of PHYTOPATHOLOGY as a practical and economical publication in which to advertise.

PHYTOPATHOLOGICAL CLASSICS

The editors of "Phytopathological Classics" reported through H. H. Whetzel that the first number, a translation of the paper of Fabricius on "Attempt at a Dissertation

on the Diseases of Plants," is now on sale. It may be secured from the Business Manager of PHYTOPATHOLOGY as long as the supply lasts, at 40 cents a copy. Exactly 100 copies were sold during the meetings.

REPORT OF THE ADVISORY BOARD

The personnel of the board for 1926 is as follows: N. J. Giddings, chairman, representing the Northeast; F. C. Meier, secretary, representing the U. S. Department of Agriculture; M. W. Gardner, representing the Midwest; B. B. Higgins, representing the South; S. M. Zeller, representing the West; J. E. Howitt, representing Canada; and F. D. Fromme and M. F. Barrus, representatives at large. The Council appointed J. G. Dickson to succeed M. W. Gardner, and E. L. Nixon to succeed N. J. Giddings. At a meeting of the Advisory Board, F. D. Fromme was elected chairman for 1927.

Summer Meetings. A summer meeting of the Society was held on Saturday, August 21, at Ithaca, New York, at the time of the meeting of the International Congress of Plant Sciences. It rained hard all day so that only a few persons went on the trip, which included a visit to the pathological greenhouses at the New York State College of Agriculture and to the vegetable gardens and the virus disease plantation on the college farm.

At the annual meeting of the board it was decided to accept the invitation from Ohio to hold the summer conference in that state. Dr. H. C. Young, of the Ohio Agricultural Experiment Station, was appointed chairman on arrangements.

The Arthur Rust Project. The rust book is nearly completed, only one chapter remaining to be written. The funds for the completion of the project appear to be sufficient.

Investigations Conducted Through the Crop Protection Institute. (From a report submitted by W. C. O'Kane, chairman of the Board of Governors.) Activities of the Crop Protection Institute in 1926 have continued along substantially the same lines as those followed in the last four or five years. The institute is administering various investigational projects, as noted below, and is publishing the results where conditions warrant publication. The scope of its work is gradually increasing, as more and more industrial organizations entrust it with funds for the prosecution of research.

Direction of the Institute's activities rests with the Board of Governors, three of whom are named by the American Association of Economic Entomologists, three by the American Phytopathological Society, two by the Association of Agricultural Chemists, and one by the National Research Council. The members of the Board for the year just closed have been as follows: Prof. P. J. Parrott, Geneva, N. Y.; Dr. N. J. Giddings, Morgantown, W. Va.; Dr. I. E. Melhus, Ames, Ia.; Prof. W. C. O'Kane, Durham, N. H.; Dr. M. F. Barrus, Ithaca, N. Y.; Mr. W. P. Flint, Urbana, Ill.; Mr. Paul Moore, Washington, D. C.; Dr. B. L. Hartwell, Kingston, R. I.; Dr. H. J. Patterson, College Park, Md. The officers of the board for the past year have been W. C. O'Kane, chairman, Durham, N. H., and Paul Moore, secretary, Washington, D. C.

Active projects on hand are as follows (only projects of especial interest to plant pathologists are reported below):

The copper investigations, which were begun in April, 1925, with funds supplied by copper refiners, have been continued with headquarters at the Boyce-Thompson Institute at Yonkers, N. Y., with Dr. Frank Wilcoxson as investigator. Some interesting new copper compounds have been worked out which promise to be of value as fungicides and which will be tested in the field the coming season.

The crown gall studies supported by the American Association of Nurserymen and an independent committee of nurserymen, with additional funds supplied by the Iowa

State College and the University of Wisconsin, have continued with headquarters at Madison and Ames. The investigators employed have been: Dr. A. J. Riker, Mr. L. W. Boyle, Dr. J. H. Muncie, and Mr. M. K. Patel. In the course of the present year an appropriation assigned to the Department of Agriculture became available for certain phases of this work and the organization of the work was rearranged accordingly. The American Association of Nurserymen again voted funds which the Institute is administering.

The study of seed-borne parasites, supported by funds supplied by the Bayer Company, Inc., with Dr. C. R. Orton in charge, continued through the year, with headquarters at the Boyce-Thompson Institute.

A renewal of contract was made with the Miner Laboratories of Chicago, on behalf of the Quaker Oats Company, for a study of furfuramid and related compounds. Necessary funds have been made available and the work will start early in the coming calendar year, with headquarters at Ames, Iowa.

Publications of the year include the following: Bulletin 8, "Colloidal Sulphur: Preparation and Toxicity"; Bulletin 9, "Suggestions on the Preparation of Apple Grafts"; Bulletin 10, "The Effectiveness of Various Fungicides in Controlling the Covered Smuts of Small Grains."

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Representative on American Type Culture Collection. C. L. Shear presented the following report:

"The culture collection which is in charge of Doctor Weaver, of the John McCormick Institute in Chicago, under the direction of a committee composed of representatives from the bacteriologists, plant pathologists and other societies, has shown satisfactory development during the past year. The collection now includes pure cultures of about 1,200 different organisms, about 700 bacteria, 175 yeasts, 68 Actinomyces and 250 other fungi. About 2,715 cultures have been distributed during the year, and receipts from the sale of cultures have amounted to \$2,332.75. A printed catalogue of the available cultures is now in process of publication, and will soon be available for distribution. It is hoped that all who are interested in pure cultures of microorganisms will avail themselves of this opportunity to secure them. More funds are needed for the development of the project. The Association of American Bacteriologists is contributing \$400 per annum, and it is suggested that, if the American Phytopathological Society has any funds which might be available for this purpose, a contribution would be very helpful in carrying on the work. It is also suggested that all persons having pure cultures of new or interesting microorganisms, especially fungi, send samples of such cultures in order that they may be added to the collection and made available to other investigators."

Committee on Public Information Service. W. A. McCubbin and F. C. Meier submitted the following report which was adopted:

"A plan for getting before the public, through the press, magazines, etc., information regarding the work and results of plant pathologists over the whole country was presented to the Society at its annual meeting in Kansas City and there adopted. Under this plan there is called for a committee to direct the policy of this work; an editor and assistant editor, to be appointed by the Council upon recommendation of committee; a series of collaborators, as far as possible representing each state, whose function will be to send in all material which could be used for publication from their states; and a staff of writers who would prepare suitable press articles from the material submitted.

"The editorial staff will act as a central point of contact for the collaborators, the writers, and the press outlets, and since this staff is responsible to the Society through

the council there is thus provided direct control of the whole program and policy by the Society itself. The committee chosen consisted of: W. A. McCubbin, G. R. Lyman, and F. C. Meier; and the editorial staff nominated by them and approved by the council: editor, W. A. McCubbin, and associate editor, F. D. Fromme. The committee arranged for the services of two men who have had considerable experience in writing for the press and magazines and who, at the same time, are members of the Society so that their viewpoint and the accuracy of their statements could be relied upon. Two other men of similar training and experience have been located to be called upon if need arose. By correspondence and personal visits of the staff, an assured outlet for all suitably prepared material was provided for through Science Service.

"The committee further prepared a list of men in the various states who would probably be most satisfactory as collaborators, and these were canvassed by circular as to their willingness to undertake this part of the work. To the 48 letters thus sent out, 29 replies were received; 27 agreed to assist and two were not in sympathy with the movement. The general tone of the replies was distinctly favorable to the scheme and expressed a willingness to help it. The committee then sent out a sheet for the guidance of the state collaborators, indicating the general program, outlining the method of procedure that was to be followed, and giving in some detail the type of material that would be acceptable from the collaborators together with the additional information that would be useful in write-up work.

"The above report indicates the steps that have been taken to date to put this plan into effect. This committee urges on the members of the Society as well as on individual collaborators to give this plan earnest support, especially during the initial period. Your committee feels assured that when the scheme has become firmly established the obvious benefits that will come to the author, the State, and the institution from widespread, accurate and sympathetic publicity will create a momentum which will render its success certain as the years go on."

Resolutions Committee. The chairman, L. M. Massey, presented the following resolution which was unanimously adopted:

Resolved: The American Phytopathological Society wishes to express its appreciation to the University of Pennsylvania, especially to the Committee on Local Arrangements, for the excellence of the arrangements provided for our meetings; and to the citizens of Philadelphia for their kindness and hospitality during our visit.

Auditing Committee. The Auditing Committee reported through its chairman, G. K. K. Link, as follows:

The Committee on Audits has examined the books of the Secretary-Treasurer of the American Phytopathological Society. We find the assets, liabilities, receipts, expenditures, and balance as reported by the Secretary-Treasurer to be correct. The committee takes this occasion to compliment the Secretary-Treasurer for the condition of the books.

Permanent Committee on Necrology. The formal report of this committee concerning the three members of the Society who died during 1926, George Richard Lyman, Richard Chambers Walton, and Harold Wakefield Fitch, will be found at the end of this record. It was the last order of business and the ceremony in connection with the making of the report was impressively conducted by Doctor Jones for the committee, which consists of Haven Metcalf, G. P. Clinton, and L. R. Jones.

ACTION OF THE COUNCIL

In addition to making the appointments of officers mentioned at the beginning of this report, the Council considered a project of mycological surveys in foreign countries proposed by J. R. Weir and submitted to the Society by the National Research Council

for an opinion. The following motion with regard to it was passed by the Council and approved by the Society:

"It is believed that information of the type to be secured by mycological surveys will be extremely valuable. The general principle of such surveys in foreign countries is good. The Council has not had time to consider the plan proposed for conducting these surveys in sufficient detail to express an opinion on this part of the project."

The Council also passed a motion—

"That the American Phytopathological Society recommend to the Council of the American Association for the Advancement of Science that they invite the Southern Association of Agricultural Workers to meet with them next year at Nashville."

It was voted also to appropriate a sum sufficient to cover the cost of the testimonial gift presented to the guest of honor on the occasion of the annual dinner.

MISCELLANEOUS BUSINESS

Dr. L. R. Jones discussed the question of securing disease-free seed and made the following motion, which was passed:

Moved: That the American Phytopathological Society recognizes the need of better development and increased use of methods for encouraging plant improvement, especially with respect to the development or discovery of disease-resistant strains through breeding or through collection and study of varieties already in existence, and with respect to the production of disease-free seeds whether by disinfection, field inspection, certification or other means. Said Society hereby authorizes its President to appoint a special committee to cooperate to this end with representatives of the American Seed Trade Association, the National Cannerymen's Association, and any other representative organizations interested in the matter.

The President appointed on this Committee on Plant Development, L. R. Jones, chairman, M. F. Barrus and M. W. Gardner.

The reports of officers and committees as given in the preceding pages of this report were adopted, as was also the Secretary's report of the last annual meeting (Phytopath. 16: 647-663. 1926).

At the annual dinner, the vice-president, H. B. Humphrey, presented a report for the Committee on an Official Seal for the Society. Several designs that had been submitted were thrown on the screen, and an appeal was made for more suggestions and sketches from the members. It is hoped that the members will heartily cooperate in this matter with the result that a dignified and appropriate seal may be selected at the next meeting.

It was voted, on a motion by A. G. Johnson, that an expression of appreciation be sent to the Graduate School of the University of Minnesota for their financial assistance to PHYTOPATHOLOGY during the past two years.

The next regular meeting of the Society will be held at Nashville, Tennessee, December 28, 29 and 30, 1927.

R. J. HASKELL, *Secretary*.

IN MEMORIAM

GEORGE RICHARD LYMAN, 1871-1926

George Richard Lyman was graduated from Beloit in 1894 and from Harvard in 1897. He also received from Harvard the degrees of A.M. in 1899 and Ph.D. in 1906.

From 1894 to 1896 he was Superintendent of Schools at Amboy, Illinois. During his stay at Harvard he held the Austin Teaching Fellowship in Cryptogamic Botany, and during 1900-1901 he was also Instructor in Botany at Radcliffe. In 1901-1903 he was Instructor and during 1904-1915 Assistant Professor of Biology (Botany) at Dartmouth. From 1906 to 1914 his summers were spent at Woods Hole on the staff of the Marine Biological Laboratory. During the academic year 1912-1913 he was Lecturer in Cryptogamic Botany at Harvard, taking over temporarily the teaching of Dr. Roland Thaxter. In 1915 he joined the Office of Forest Pathology at Washington, but within a few months was transferred to the Federal Horticultural Board. In 1917 he was appointed Pathologist in Charge of the newly organized Plant Disease Survey. In January, 1923, he was made Dean of the College of Agriculture in the University of West Virginia, which position he held at the time of his death.

Dean Lyman was Vice-President of Section G (Botany) of the A. A. A. S. in 1924. He was president of this Society in 1923, having previously served as a member of the War Emergency Board (1918), chairman of the Advisory Board (1919-1921), representative to the National Research Council (1919-1922), and Secretary-Treasurer (1918-1922). In this last capacity especially he placed this Society under deep obligations to him for the efficiency of his methods and his sense of organization.

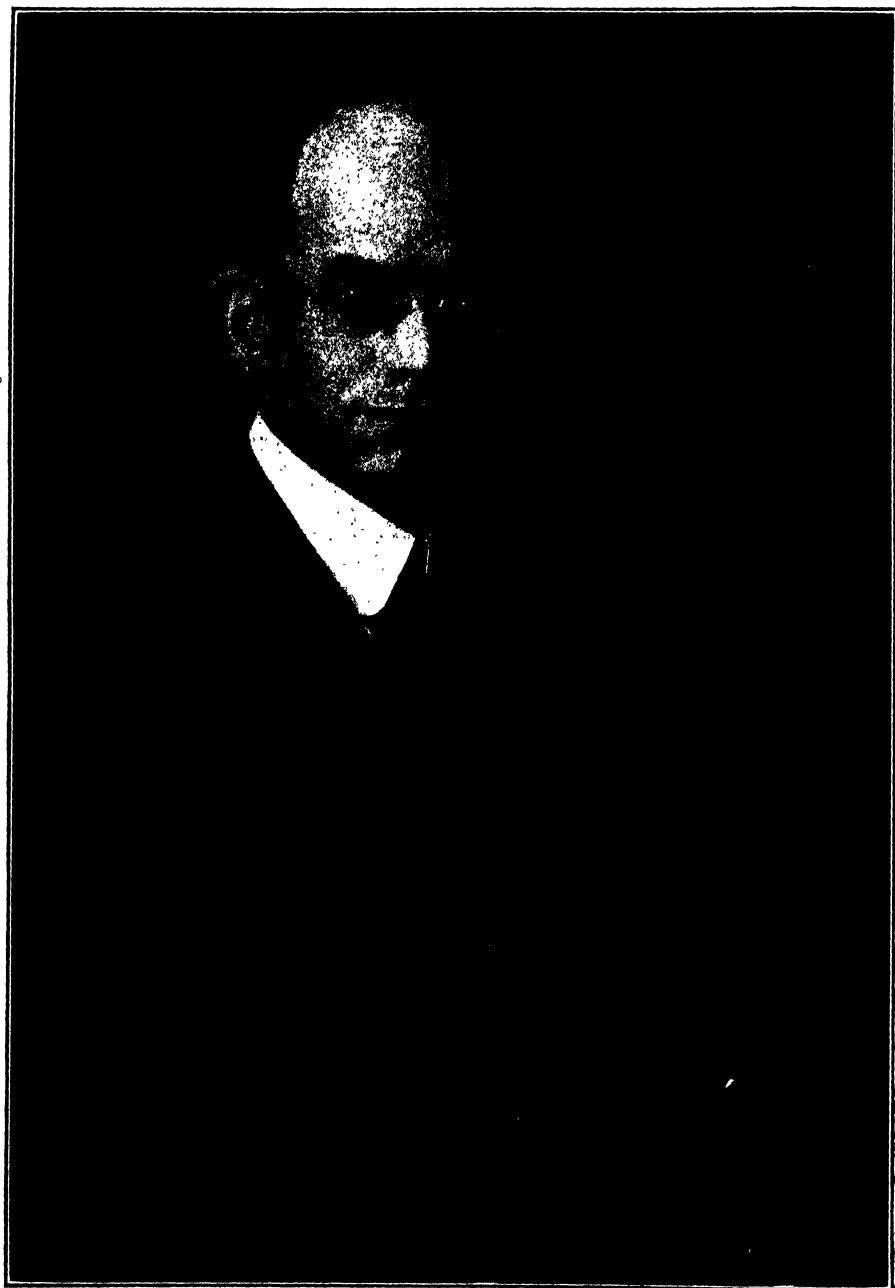
Dean Lyman was a remarkable executive of the type most needed at the present time—a harmonizer of conflicting interests. Few men have his capacity of appreciating the other man's point of view. Humorous, essentially friendly, free from cynicism, cheerful in the face of misfortune, he is sincerely mourned by a host of friends.

RICHARD CHAMBERS WALTON, 1886-1926

Richard Chambers Walton received the degree of Bachelor of Science from the Pennsylvania State College in 1911, and the degree of Master of Science in 1923. After graduation he was employed by the Pennsylvania Chestnut Blight Commission for about two and one-half years. In 1914 he went to the Ohio Agricultural Experiment Station as Assistant Botanist, where he was engaged for four years in the study of fruit diseases. When the Pennsylvania State College established a field laboratory in Adams County, Pennsylvania, in 1918, he was appointed plant pathologist in that station. Later he was promoted to the rank of Associate Professor. While connected with the Pennsylvania State College, he was concerned with the investigation of apple diseases giving special attention to frog-eye and blotch. Mr. Walton made a number of contributions to the science of plant pathology through his work in Ohio and Pennsylvania. He will be remembered by friends and associates for his excellent spirit of cooperation and for the fine enthusiasm toward his work which he maintained to the last.

HAROLD WAKEFIELD FITCH, 1897-1926

Harold Wakefield Fitch received the degree of Bachelor of Science from New Hampshire University in 1921. He had practically completed the requirements for the Doctorate of Cornell University, where he held the Herman Frasch Fellowship in Plant Pathology. At the date of his untimely death he was a member of the scientific staff of the Niagara Sprayer Company. In his chosen field of applied plant pathology he was a man of great promise.



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GEORGE RICHARD LYMAN

1871-1926

REGINALD H. COLLEY AND W. H. WESTON, JR.

Recording the main facts of a man's existence, and his passing, is a relatively simple task. To interpret and express the influence which the personality moving in that existence had on his fellows is incomparably more difficult. Influences are elusive and intangible; kindness, sincerity, and unwavering adherence to ideals too soon become shadows of the memory; they are never adequately portrayed.

George Richard Lyman was born on December 1, 1871, at Lee Center, Illinois, and died at Baltimore, Maryland, on June 7, 1926. His parents were George A. and Mary E. (Jones) Lyman. On June 23, 1903, he was married to Frances E. Badger, of Amboy, Illinois. Mrs. Lyman and their one daughter, Mavis, and his aged mother, survive him.

During his boyhood Dr. Lyman lived on a grain and stock farm in Northern Illinois. When 18 years old he went away for further training than the local schools could give; and in 1894 he received his A.B. degree from Beloit College. He then became superintendent of schools at Amboy, Illinois, for two years. He resigned to do graduate work at Harvard University. After securing an A.B. degree in 1897 and an A.M. in 1899, he continued his studies there while acting as Austin Teaching Fellow in Cryptogamic Botany from 1897 to 1900, and as Instructor in Botany in Radcliffe College in 1900-1901. In 1901 he went to Dartmouth College as Instructor in Botany; and in 1904 he was made Assistant Professor of Biology (Botany). He held this title at the time of his resignation from Dartmouth in January, 1915. In June, 1906, he received a Ph.D. degree from Harvard. During the school year 1912-13 he was on leave from Dartmouth, and, in Dr. Thaxter's absence, took charge of the work in cryptogamic botany at Harvard, with the title of Lecturer. His summers from 1906 to 1914 were spent at the Marine Biological Laboratory, Woods Hole, as one of the staff of instructors in the course on the morphology and physiology of the Algae.

He entered the service of the United States Department of Agriculture on February 1, 1915, for the first month and a half dividing his time between the Office of Forest Pathology and the Federal Horticultural Board. On March 16 of the same year, however, he was transferred to a full time position as a pathological inspector in the Federal Horticultural Board; and in this service he took an increasingly valuable and responsible part until July 31, 1917, when he assumed the direction of the newly-created Plant Disease Survey.

On January 15, 1923, he resigned from the Department of Agriculture to become, on January 21, Administrative Head in Charge of the Agricultural Experiment Station, the Division of Agricultural Extension, and the instructional work in the College of Agriculture of West Virginia.

Dr. Lyman was a member of Beta Theta Pi and of Phi Beta Kappa. He was elected to the American Association for the Advancement of Science (Sections G and O) in 1906, and made a Fellow in 1909; he was vice-president of Section G (Botany) in 1924. He was a member of the Botanical Society of America, the American Society of Naturalists, the Botanical Society of Washington, and the Washington Academy of Sciences; he was a Fellow of the American Academy of Arts and Sciences.

His membership in the American Phytopathological Society began in 1914; and he became a life sustaining member in 1920. He was elected secretary-treasurer of the society and business manager of PHYTOPATHOLOGY at the Baltimore meeting, December, 1918, and served until December, 1922. Dr. Lyman took office at an exceedingly difficult time; war prices prevailed for printing, as well as everything else, and were still mounting. The journal was going into debt an increasing amount each year; its poor financial condition caused him a great deal of worry. His gratitude when the members of the Society contributed practically half of the indebtedness facing the Society at the time of the Chicago meeting was a deeply personal thing which indicated how close was the bond between the Society and himself. A change in the methods of handling the journal had become imperative, and after much consideration he made the change in 1920. It resulted in a much improved condition for PHYTOPATHOLOGY but it threw on him additional duties connected with the management of subscriptions, advertising, and sales, which up to that time had been handled by the publishers.

The War Emergency Board originated at the Pittsburgh meeting, December, 1917; and he was the representative on the Board from the Department of Agriculture. He put a great deal of time and effort into the work and was one of the most useful members of the Board. His counsel was always sought, and the members soon discovered his ability, good judgment, and wisdom. When the War Emergency Board was succeeded by the Advisory Board, he was elected chairman. He also served as the Society's

representative in the Division of Biology and Agriculture of the National Research Council from 1919 to 1922.

He was president of the Society for 1923.

Thus can be set down the chief mile stones in his journey; those of us who walked with him even a short space have a vivid recollection of the man in him that made the journey unforgettable. Very early in his career Dr. Lyman won the confidence of his associates. In college he took his place as a leader in the affairs of his fraternity and in the activities of the college as a whole. The same qualities which had characterized him from the first developed steadily throughout his work as student, teacher, investigator and executive. His personality was singularly distinct; and the elements which made it so were many. Courteous simplicity, open honesty, and an incredible lack of cynicism were combined with gentle humor, kindness, patience, and sincerity. These qualities were the foundation on which he builded his success and influence.

His deep interest in his subject and his painstakingly clear presentation of it naturally aroused in certain of his students an eagerness for further study in some phase of botany. As the years went on, an appreciable number of men were thus led into their life activity. Some devoted themselves to teaching, some to government service, some to forestry, and some to commercial botany. They were as different as the phases of work they had taken up and as the various schools of training in which they had pursued their graduate work; but all of them shared one thing in common—an enthusiasm which Dr. Lyman had transmitted to them and awakened in them, and a love and respect for the man who had started them in their profession.

He was one of the clearest and most effective lecturers we have ever heard. It required considerable mental and manual agility to keep up with him, for one could not safely omit as unimportant any point which he brought forward; his notes had been culled of the uncontributive and unimportant before he started to talk. Although essentially patient and tolerant, he could be effectively severe when necessary. In the laboratory he taught his students the value of adequate records and impartial interpretation, insisting on the utmost attainable accuracy in observation and representation of the things studied.

He was not restricted to books and laboratory, for he knew and taught plants as they grew. By nature a keen observer, collector and field investigator, he had studied widely different groups of plants—algae, fungi, trees and flowering plants—in their natural surroundings; and he made them all vital and interesting in his fascinating courses. The field trips which he conducted were by no means picnics. Rather, during them a great deal of very effective teaching was done in the open. Despite a general attitude of

informality, much was demanded and much was accomplished in response to his demand. Actively he led, here, there, and everywhere at once, scooping a bottle of slimy mixture from a green and odorous pool, pointing out the gross differences between a *Russula* and a *Lactarius*, suggesting aptly that an old log had better be rolled over—there might be some fine Myxomycetes on its underside.

There was a directness about his methods which accomplished pedagogical wonders within the hours available for such work. At times a puckish twinkle in his eyes would reveal the saving and sympathetic humor underlying his academic seriousness. “This, gentlemen, is *Arisaema* and, as you note, the corm is especially developed for storage. Would you, Mr. Husis, care to taste it?” He would, and did. After all there was something about *Arisaema* which made one remember it! Perhaps it was evolved just to break up the tenseness toward the end of a field trip. In any case the demonstration was successful.

In his outdoor work he always showed himself a thorough sportsman. He was an unusually able walker. Many an athlete, swinging a mile or two back to the college at a four-mile-an-hour rate, after a field trip that had covered several miles through rough and rugged country, learned to look at the spare but vigorous figure with increasing respect. He was never unduly censorious if a convenient snow-ball in winter or a snake or toad in spring were slipped deftly into the gaping collar or pocket of the intent and unwary by those whose high spirits led them to activities that embellished the more serious intellectual work in hand.

The quality so essential in a teacher of being patient with those who were just stepping out on the path along which he had gone so far was carried into all phases of his life's work. In dealing with hard-headed individuals or with pompous gentlemen of more eloquence than knowledge, who sometimes gathered to protest against various measures, his patience and understanding kindness were always unaltered and undiminished. Dr. Lyman was distinguished by his obvious sincerity. If he urged a student to improve his drawing, it was because he truly believed that the student should better himself; if he urged the destruction of a shipment of valuable but dangerously infected plant material, the disgruntled investigator for whom the shipment was destined realized that there was no false pretense back of his recommendation; if he appealed for greater funds for carrying on some phase of departmental work, there stood out in his appeal—even in a letter or memorandum—the unselfish conviction that the appropriation was necessary for the betterment of the service. All who came in contact with him felt this sincerity, and, as was inevitable, it communicated itself to others; no one was associated with him in any phase of academic or departmental activity without being in some measure influenced by it.

In Dr. Lyman's work as an investigator there may be traced the same qualities that characterized him as a teacher. His doctor's thesis represents a painstaking, conscientious piece of investigation in a very difficult field, where there was so little promise of reward that a less earnest or persevering worker would have given up, discouraged, long before the first results were obtained. Moreover, its completion was materially retarded by the heavy load of teaching which he assumed at Dartmouth in 1901. In spite of such obstacles it was finally made ready and published in February, 1907. In the years that followed, only a few occasional papers appeared. The thesis on the polymorphism of the Hymenomycetes, a very able piece of work, remains his only major contribution to mycological literature.

During his academic service his creative efforts went into the preparation of his lectures, his reports, his plans of organization or procedure, outlines of the course work, and keys for the identification of fungi, flowers, shrubs, and trees. His key to the trees around Hanover, N. H., a small pamphlet used in one of his classes at Dartmouth, is still one of the most practical and useful manuals of its kind which we have ever encountered. One of us still treasures a manuscript of some 30 pages of certain parts of Saccardo's keys, which he painstakingly copied years ago as an aid in his study and teaching. Lantern slides, material, and charts used by him at Dartmouth and Harvard still bear his neatly written labels; schedules for laboratory work and field trips are pinned on the wall of his office at Woods Hole—mute evidence of the care that he took in even the most minute details of his work.

The record of Dr. Lyman's years in the Department of Agriculture is so recent, his success as an organizer and an administrator there are so well known that little need be written here. There was a vast difference between the duties of a quiet academic position and the executive work of the Federal Horticultural Board which involved varied contacts not only with investigators, but also with growers, shippers, and business men. An unusual amount of seasoned executive ability appeared to be required. At first sight his success and progress seem surprising. A relatively short time showed clearly how effectively the teacher could adapt himself to his new surroundings. The ability which had enabled him to keep pace with the expanding department of botany at Dartmouth enabled him also to meet the demands made on him by the rapid development of the pathological inspection work, and by the active growth of the Plant Disease Survey.

He had remarkable ability as a mediator and pacificator. For example, his efforts in the dispute over the proposed location of the potato wart quarantine zone in Pennsylvania in 1920 straightened out what might have been

a very serious situation. Conditions which irritated others aroused in him a sort of amused tolerance that rendered him particularly effective in difficult situations. Disputes, professional or quasi-political, were quietly settled; his knowledge of the actual scientific facts involved, combined with liberal common sense and human appreciation of the widely varied points of view which were represented, won over even the most unreasonable.

Under his able direction the Plant Disease Survey became an active organization of collaborators who reported plant disease conditions in their respective regions and cooperated with the Department in special survey projects. The machinery for mapping epidemics and for estimating their cost to the country was set in motion, and kept running with great efficiency. In a few years, he who had held a relatively isolated professorship had built up a phytopathological organization of nation-wide importance, and become an authority on the problems with which it dealt. There can be no better expression of appreciation of this service than the following paragraph from the letter written by Dr. W. A. Taylor, chief of the Bureau of Plant Industry, to acknowledge Dr. Lyman's resignation:

"The development of the Plant Disease Survey work has required unusual tact, patience and perseverance, qualities which we have all been gratified to recognize as possessed by you and stimulated in your associates through the influence of your example. It has been a class of work of pioneer character in the pathological field and we feel that you have rendered a service of very great value, not only to this department, but to science generally, where too frequently in the past individualistic tendency has prevented or unfortunately retarded constructive cooperative effort."

With his call to West Virginia University there came to Dr. Lyman opportunity to continue his activities as an executive and administrator in an even more varied field and to take up again his academic work as a teacher and leader. That the three years allotted to him there were productive ones is evidenced by the esteem in which he was held by the college and by the extension and experiment station staffs. That his work in the future would have been even more valuable and important is certain.

The news that Dr. Lyman was in poor health reached us in November, 1925, just at a time when we were visioning for him a hope picture of a particularly bright future. The serious nature of his illness became apparent after an operation at Sibley Hospital, Washington, on December 14. A second operation at Johns Hopkins Hospital on May 6, 1926, served only to confirm the diagnosis of cancer and to reveal the hopelessness of his case. With characteristic fortitude and patience he went through the days that followed. On June 6 he appeared, as one friend has written us, "so like Doctor Lyman of old"; but very early on the morning of June 7 the un-

equal struggle ended. Funeral services were held on June 10 at Morgantown, West Virginia, and some days later the interment took place at Dixon, Illinois.

It is impossible to write of him without mention of what was so large, so vital, and so fundamentally a part of his life—his love for his family. His utter considerateness toward Mrs. Lyman and toward his daughter Mavis was a keynote in his character. Their suffering was his and their pleasure his deepest enjoyment; in their difficulties or triumphs they were inexpressibly close to his heart; invariably it was his custom to place them and their happiness before himself and his own interests.

His botanical contributions may be overshadowed by the results of more comprehensive investigations; his achievements in various offices at Washington may be superseded by the activities of others; but some measure of his ideals will persist and extend the influence of his personality through his students and associates.

To the best of our knowledge the following is a complete list of Dr. Lyman's publications:

Culture studies on the polymorphism of Hymenomycetes. *Proc. Boston Soc. Nat. Hist.* **33**⁴: 125-209. 1907.

Keys to the trees of Hanover, New Hampshire, based on winter characters and on leaves. Issued by Dartmouth College, 1909.

The trees and shrubs of Hanover, New Hampshire. Issued by Dartmouth College, 1911.

Keys to the fungi of Hanover, New Hampshire. Privately issued, 1912.

Outlines of cryptogamic botany. Privately issued, 1914.

The native habitat of *Spongospora subterranea*. *Science* **42**: 940-941. 1915.

The need for organization of American botanists for more effective prosecution of war work. *Science* **47**: 279-284. 1918.

The relation of phytopathologists to plant disease survey work. *Phytopath.* **8**: 219-228. 1918.

The unification of American botany. *Science* **49**: 339-345. 1919.

The Advisory Board of American Plant Pathologists. *Phytopath.* **9**: 202-206. 1919.

Potato wart—a dangerous new disease. U. S. Dept. Agr. Circ. 32. 1919.

Potato wart. U. S. Dept. Agr. Circ. 111. 1920.

Reports and summaries on distribution, severity, etc., of plant diseases in the United States. *Plant Disease Bulletin*. U. S. Dept. Agr. 1917-1923.

Reports of the Annual Meetings of the American Phytopathological Society. *Phytopath.* **10**: 264-271. 1920; **11**: 194-204. 1921; **12**: 195-204. 1922; **13**: 188-198. 1923.

PHENOMENA ASSOCIATED WITH THE DESTRUCTION OF THE CHLOROPLASTS IN TOMATO MOSAIC¹

HELEN SOROKIN²

INTRODUCTION

Despite the fact that leaf mottling is one of the most conspicuous symptoms of the mosaic diseases, very little attention has been paid in modern literature to the actual process by which the chloroplasts are destroyed in the chlorotic regions of mosaic leaves. In the general search for the pathogene of this peculiar disease, various pathological cell inclusions, like protoplasmic bodies attached to the nuclei, or various motile "protein bodies," attracted most of the attention of investigators.

The present paper describes certain pathological inclusions found in mosaic affected cells of tomato leaves, but its main purpose is to describe the disintegration of the chloroplasts, and to try to explain this process from the point of view of changes which might occur in such a colloidal system as a living cell.

The observation of living material has been the principal method of the investigation. The fixing of free-hand sections on the slide under the microscope was practiced to a considerable extent, and cytological technique served only for some supplementary purposes, as well as for the histological observations. As a fixing reagent in this latter case, Merkel's fluid was found to be the most suitable. The sections were stained with haematoxylin and with safranin and gentian-violet.

HISTOLOGICAL CHANGES IN MOSAIC TISSUE

Woods (34) and Iwanowski (12) established the fact that there are definite and typical changes in the structure of mosaic leaves of tobacco. The most conspicuous of these were the failure of the tissues in the chlorotic

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² The investigation was undertaken as a cooperative project between the Section of Plant Pathology and the Section of Plant Physiology.

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regions to differentiate into palisade and spongy parenchyma, and the development of more or less isodiametric cells. Histological studies on tomato were made by Westerdijk (33), Melchers (24) and Dickson (4). Westerdijk did not find any striking differentiation between the yellow and the adjoining green or healthy areas in mosaic tomato leaves, except that the yellow regions were sometimes only slightly thinner. Somewhat similar results were obtained by Melchers. Dickson, on the contrary, showed that distinct histological abnormalities occur in severe cases of mosaic. Sections through the chlorotic regions showed the presence of cuboidal palisade cells, while sections through the dark areas of mosaic tomato leaves had perfectly developed, elongated palisade cells. In less severe cases of the disease the histological changes were not so pronounced, but still there was a reduction in the length of the cells. Furthermore, Dickson (4) brought forward some additional evidence for the general conclusion that the lighter green areas in many mosaic diseases are characterized by hypoplasia of palisade and spongy parenchyma, as well as by the reduction of the intercellular space volume in these tissues.

The writer's observations of the histological changes in tomato leaves affected with mosaic are in complete agreement with the conclusions of Dickson. A section through a decidedly chlorotic region of a young leaf in a severe stage of mosaic disease is represented in Plate XII, A. All cells are nearly isodiametric; there is no differentiation into palisade and spongy parenchyma; the intercellular space volume is very limited; the epidermal cells are hardly distinguishable from the parenchyma cells. The chlorotic regions in less severely diseased leaves are characterized by the fact that the first row of parenchyma cells are slightly longer than those of the other three or four rows. In other respects the condition is similar to that which is represented in Plate XII, A. As to the cell contents, almost all of the essential components of a normal cell are lacking or modified. The nuclei are often absent or only about half of their normal size. Chloroplasts are absent entirely in a great number of the cells. In other cells few modified plastids could be observed. Groups of transparent vesicles often occupy the cell, or it may be vacuolated. The deep green portions, on the contrary, have normally developed palisade cells (Pl. XII, B). The spongy parenchyma and intercellular spaces are present and the epidermal cells are somewhat hypertrophied. A transitional zone between the two described regions is shown in Plate XII, C. In many of the cells of this region the chloroplasts are absent or replaced by transparent vesicles. In other cells the plastids are present. There often is a granular substance in cells which are devoid of the normal cell components.

PREVIOUS STUDIES ON THE CAUSE OF CHLOROSIS IN MOSAIC TISSUES

The studies of different authors on the structure of the chloroplasts in chlorotic regions of different plants affected with mosaic have mostly been made on fixed material. A generally accepted opinion is that the chloroplasts are fewer, paler and smaller in the chlorotic regions than in normal tissues. Köning (14) was the first to call attention to the disorganization of chloroplasts in mosaic tobacco plants. He also observed a general disorganization of the leaf tissues. Westerdijk (33) says that in yellow areas of the mosaic tomato leaves the chloroplasts are yellowish, slightly smaller than normal, and contain but little starch. Again, Melchers (24) found no difference in the number or size of the chloroplasts from the chlorotic and green areas in tomato leaves. Doolittle (5) found that the chloroplasts in the cells of cucumber affected with mosaic were smaller than in the normal cells, and were often pressed so closely to the walls of the cell as to be almost invisible. Kunkel (15) explains the occurrence of the lighter regions in corn mosaic as due to the failure of those portions to develop the normal green color rather than to a fading out of the green color after it has been produced. Kunkel (17) states that it is possible to observe the chlorotic areas in the process of formation in mosaic sugar cane. It consists in the coalescence of scattered, small chlorotic spots into a larger spot. A decrease both in number and in size of the chloroplasts has been observed by Cook (1) in the chlorotic regions of mosaic sugar cane. He explains this condition as being a result of under-development rather than disintegration. Rawlins and Johnson (30) state that the chlorotic appearance in mottled mosaic leaves of tobacco is due to a failure of the palisade cells of these plants to elongate.

Iwanowski (12) studied the changes in the chloroplasts of living cells in the chlorotic regions in mosaic tobacco. He found that the chloroplasts were very scarce, often were either swollen or liquefied or changed to peculiar vesicles with a thin green rim. These vesicles sometimes occupied the whole cell (Iwanowski's fig. 7). Dickson (4) made numerous observations of the chloroplasts in living cells in the chlorotic regions of the different mosaic plants. His general conclusions are that the chloroplasts are fewer, paler, often broken down, coalesced and degenerated. According to him, this latter process is as follows: The chloroplasts lose their green color and break up into many small, hyaline granules of varying size, which move rapidly. The bodies become brownish when treated with iodine-potassium iodide.

THE DESTRUCTION OF CHLOROPLASTS IN MOSAIC TISSUES

In the present investigation, a process of the actual dissolution of the proteins of the chloroplasts has been observed in the chlorotic regions of the

diseased leaves. This process was accompanied by peculiar changes in the general aspect of the plastids. For the observation of the above mentioned phenomenon in the living condition, thin sections were made from the surface of the transitional zone between the green and chlorotic regions of mosaic leaves. The sections were cut parallel to the leaf surface in such a way as to leave the epidermal cells and the first row of the parenchyma cells untouched. These sections were mounted in water, and were observed by focusing through the epidermal cells. Apochromatic, oil immersion lenses were used. A camera lucida drawing of part of one of these sections is represented in Plate XIII, A. In the two cells at the extreme upper left of this drawing there are numerous apparently healthy chloroplasts. In a homogenous stroma are included granules and droplets (Pl. XIII, C, 1). Hyaline bodies, which were surrounded by a halo (Pl. XIII, C, 2), were found in certain chloroplasts from the green area, and in very many chloroplasts from the transitional zone between the green and chlorotic areas. The bodies measured about 2μ in length and were stationary. In certain other chloroplasts the halo was absent and the hyaline bodies were actively revolving (Pl. XIII, C, 3). If viewed from one side, the bodies appeared like elongated crystals with one edge somewhat pointed and the other slightly rounded (Pl. XIII, E, 1). There was a definite black stripe extending along the long axis of the body. The stripe connected two small black points (Pl. XIII, E, 1, 2, 4, 5, 6, 8, 9). Some of these bodies, after they turned around the long axis, showed a cap-shaped structure adherent to the whole length of the body (Pl. XIII, E, 6). The external edge of the additional structure was either semi-circular or showed an angle. The black stripe was not visible if the body was viewed from the cap-shaped side. In the transitional zone of a mosaic leaf which contained chloroplasts with the hyaline motile bodies there were peculiar transparent spheres attached to the chloroplasts. Careful examination of the material revealed different stages of the dissolution of the chloroplasts and formation of the transparent spheres. The intermediate phases of this process are illustrated in Plate XIII, C and D. In the earliest stage, a blister-like protrusion begins to appear along one side of the chloroplast (Pl. XIII, C, 4). The moving hyaline bodies, two or more in number, at that stage of the formation of the protrusion, are visible only in the chloroplast. In some other chloroplasts the blister-like spheres were about half the size of the plastid (Pl. XIII, C, 5). When the spheres are larger than the plastid, the actively motile bodies are no longer seen in the chloroplasts, but they move about within the spheres. Finally the chloroplast disappears entirely and the sphere reaches a size of about 10μ in diameter. Numerous spheres may occupy the entire cell, each sphere containing actively moving hyaline bodies. Successful perma-

nent preparations of the spheres and late stages of the dissolution of the chloroplasts in the cells of the chlorotic region were obtained by fixing the material with one per cent picric acid and staining with aniline blue. A camera lucida drawing of such a preparation is represented in Plate XIII, B. The disintegrating chloroplasts appear brown; the spheres are almost transparent and slightly greenish. The hyaline bodies inside of the spheres are not fixed well enough by this method to be distinct. By treating the leaves containing rather small chlorotic and green areas by the above described method, it is easy to obtain excellent preparations showing the difference in the chloroplasts of the diseased and healthy tissues. The chloroplasts of the green area stain bright green (yellow after picric and blue after aniline blue) while the chlorotic region contains the brownish remains of the chloroplasts and the transparent spheres. The brilliant green spots on the slide are easily distinguishable from the brownish-yellow spots.

PHYSICAL AND CHEMICAL PROPERTIES OF THE BODIES ASSOCIATED WITH THE DESTRUCTION OF CHLOROPLASTS

For the study of the physical and chemical properties of the chloroplasts and the spheres it seemed desirable to isolate both of them from the other cell contents. This was accomplished by tearing the epidermis and the palisade parenchyma with needles under a dissecting binocular and mounting the resulting mass of chloroplasts and spheres in distilled water. By regulating the amount of water carefully, it was possible to observe the chloroplasts and the spheres on the same preparation for several days. To make the spheres more distinct, some dilute solutions of stain were applied to the mounted material. Of the typical vital stains, only thionin stained the spheres slightly. A dilute solution of Giemsa stain applied *in vivo* stained the spheres light violet. Dilute water solution of gentian violet proved to be very satisfactory. The chloroplasts appeared dark purple and the spheres lavender, while motile bodies did not take the stain.

Mounts of the spheres and chloroplasts from the transitional zone between chlorotic and green areas of a diseased leaf, which were prepared and stained *in vivo* as above described, were photographed with a moving picture camera. In Plate XIV, A are represented enlarged prints from the positives. The majority of the chloroplasts exhibit the phenomenon of dissolution of the mass of the body. Various transitional stages of this process can be found in the accompanying illustrations. The white ellipsoidal bodies on the print are the chloroplasts. A considerable number of them are healthy, while others are in different stages of the dissolution. There also are found on the print numerous complete spheres without any of the mass of the chloroplast adhering to them. The movement of the

hyaline bodies inside of the spheres is evident when the film is projected on the screen. A single print, however, does not show the bodies.

The spheres are very regularly spherical in shape and vary in size from 8-10 μ in diameter. In living condition they seem to be homogeneous in structure, transparent and slightly reflecting. There usually are two or three moving bodies inside of those spheres to which a considerable amount of the mass of the chloroplast still adheres. When the chloroplasts have completely disappeared, there are more moving bodies inside each of the spheres, the average being from 5 to 8 and occasionally as many as 15.

In iodine-potassium iodide solution the spheres become yellowish-brown, while the moving bodies inside of them become a deeper brown. This reagent does not stop the movement of the hyaline bodies. With the addition of concentrated H_2SO_4 to the material mounted in I_2KI , some of the spheres collapse; others remain spherical or shrink only slightly and retain their brownish color. If the reagent is applied to the spheres in the tissues of tomato, the hydrocellulose reaction is very evident in the neighboring cells, but the spheres give no indication of the cellulose reaction. The reactions for fats are negative. The usual protein reactions are very indefinite, usually negative.

The spheres and the moving hyaline bodies have a peculiar reaction towards alcohol. The grades of alcohol below 85 per cent do not have a distinct effect, but 85 per cent alcohol will stop the movement of the larger hyaline bodies while the smaller ones still move, and in 95 per cent alcohol the movement of all hyaline bodies ceases. The spheres partly collapse and shrink, but they are not dissolved even in absolute alcohol. A gradual addition of acetone to material in water does not affect the spheres nor the moving hyaline bodies. When almost all the water is replaced by acetone, the spheres become especially distinct while the bodies still continue moving.

The spheres are not dissolved in 10 per cent hydrochloric acid, but a weak solution of KOH will dissolve them. The movement of the hyaline bodies is entirely stopped by the action of alkaline solutions.

It is interesting to note here that even 10 per cent KOH does not have any appreciable effect on the chloroplasts. If the chloroplasts have swollen after having been mounted in water and are then subjected to the action of 10 per cent KOH, they react in the same way as non-swollen chloroplasts. The reaction is entirely different from the reaction of the spheres to KOH. This fact indicates that the substances present in the spheres are entirely different from those in the swollen chloroplasts.

A technique commonly used in the study of the animal virus diseases was tried on the diseased tomato tissue for a comparison of the pathological cell inclusions of plants and animals. Chloroplasts and spheres from the diseased leaf were mounted in a drop of water and exposed to osmic acid

vapor for about 5 to 10 minutes. The preparation was then dried very quickly by heating in the flame of an alcohol lamp. It was then stained with basic fuchsin according to Löffler [described by Giemsa (7)], or by the usual Giemsa method without the addition of Na_2CO_3 . The spheres are rather resistant to stains and usually color along the periphery and in the meshwork inside, which is very distinct in fixed material. A deeper color of the whole structure is obtained by staining for a longer time, or by using gentian violet, for which the spheres have a special affinity. After staining with Löffler's stain the spheres appear rose-colored, the chloroplasts red, and the bodies inside of the spheres become dark, almost black-red. In Plate XV, B is represented a smear preparation of the spheres stained by Löffler's method. The spheres show a definite structure inside, which consists of granules and threads. The dark body next to the sphere is a chloroplast and the three distinct dark spots inside of the sphere are the "hyaline bodies," while the whole sphere shows peculiar lighter stained threads and granules which were not seen in the living material. It is quite evident that the granules and threads are found inside of the sphere and are not merely the shrinking of the surface of the sphere, the outlines of which are perfectly regular. In Plate XV, C is shown a later stage of the dissolution of the chloroplasts, the mass of which is somewhat elongated and spread out on the surface of the sphere. A distinct dark body is seen inside the sphere. Here also, besides the body inside of the sphere, it is possible to observe the peculiar structure. A similar condition is shown in the preparations stained with Giemsa stain (Pl. XV, D); in the upper corner to the right a healthy chloroplast is found; the two other plastids are shown in various stages of dissolution.

The chloroplasts with the adherent spheres at first suggest the well-known phenomenon of swelling which was studied long ago by Mohl (25), Sachs (31), Hofmeister (11) and others. Liebaltd (20) described sudden swelling of the chloroplasts of certain plants after they had been brought in contact with water. She found two types of swelling in the chloroplasts. The first and most common type consisted in the general increase of the size of the plastids and the appearance of vacuoles inside the stroma. The second type consisted in the formation of vesicles adherent to the chloroplasts. The conditions which determined this latter type of swelling were rather indefinite; sometimes chloroplasts of a certain species exhibited this kind of swelling, while in other cases they did not. It is quite possible that the first type was a case of real swelling, while the second may have been an entirely different phenomenon.

Zirkle (35) studied the swelling of the chloroplasts in fern prothallia and in different higher plants. His conclusions are that the phenomenon is exhibited not by the stroma of the plastid but by the substance within the

central vacuole. The stroma either remains adhering to the vacuole on one side, as in fern prothallia, or is ruptured in numerous places and distributed over the surface film of the vacuolar contents. Attempts to stain the contents of these vacuoles *in vivo* were not successful.

I observed in the same mount of diseased tomato tissue normal chloroplasts, chloroplasts with attached spheres, and transparent spheres without any amount of the stroma of the chloroplasts adhering to them. This indicates that the phenomenon is not merely a swelling of the chloroplasts as a result of contact with water. There seemed to be some factor which caused essential changes in some of the chloroplasts, as a result of which the swelling occurred. This factor was absent in the normal chloroplasts.

Before offering an hypothesis to explain the dissolution of the mass of the chloroplasts, certain physico-chemical properties of chloroplasts should be reviewed. The general substance of the chloroplast is a protein, according to most authors. As emphasized by Zirkle (35), the typical protein reactions are positive, but "not as distinct, as could be wished." Proteolytic enzymes, however, will digest chloroplasts. According to Lepeschkin (18, 19), chloroplasts and protoplasm are built in a similar way, and constitute colloidal systems. The disperse phase in the active cell is an organic fluid which consists of a loose chemical combination of proteids and lipoids. These chemical combinations imbibe water in great amount until a certain limit is reached, after which the system becomes saturated and vacuoles appear. The dispersion medium and disperse phases of chloroplasts and protoplasm are, however, chemically different.

The condition of aggregation and the consistency of the chloroplasts are changeable; sometimes they are fluid, sometimes the viscosity is so great that they become gelatinous. The method of isolation of chloroplasts from the cells, which was practised by Lubimenko³ to a great extent, indicates that normal chloroplasts are rather solid or semisolid. This method consists in soaking leaf fragments in water for several weeks until the leaf tissue decays, when the chloroplasts fall to the bottom of the dish. The microscopic studies of the chloroplasts treated in this way showed that such a treatment did not affect the plastids very seriously. Zirkle (35) also made some observations on the chloroplasts. By teasing the chloroplasts with needles he noticed that the fragments maintained their shape, from which he concluded that the chloroplasts consist of a gel rather than a sol.

From the point of view of the colloidal-chemical theory of the structure of chloroplasts, the formation of vacuoles in chloroplasts is the resultant of syneresis following the phenomenon of typical swelling or solution of water in the disperse phase. The formation of spheres in diseased tomato mate-

³ The writer was unable to locate this paper by Lubimenko. The results given are from personal information obtained from the author.

rial, on the other hand, is apparently just the reverse: it is probably a solution of the colloid in the solvent.

Fischer (6), in his excellent book, says: "The phenomena of swelling (hydration) and of 'solution' in protein gels, while frequently associated, are essentially different. Swelling is best understood as a change whereby the protein enters into physico-chemical combination with more of the solvent (water), as a change in the direction of greater solubility of the solvent in the protein; 'solution' is best conceived of as a change in the direction of greater solubility (an increased degree of dispersion) of the colloid in the solvent."

The following hypothesis seems to explain the changes in the chloroplasts of the diseased tomato plant. Observations made when teasing apart the chloroplasts in tomato plants indicate that they are normally rather solid. The first indications of a pathological condition are the appearance of hyaline bodies inside the chloroplasts and later the movement of these bodies. Previous to this movement, a liquefaction of the stroma must have taken place which may possibly be the result of the action of an organism secreting a proteolytic enzyme. This proteolytic enzyme penetrates into the chloroplast and causes the digestion of the proteins. As a result, the osmotic pressure increases inside, and water is taken in from the surrounding medium. Finally, all of the body of the chloroplast goes into solution and the sphere appears. Therefore the sphere may well represent a "membrane" resulting from the digested chloroplast in which the digestion products and water are retained, the whole being surrounded by a surface film at the interface with water, and containing moving bodies. The supposed organisms have apparently quite unusual properties, different from the properties of the generally accepted conception of an organism. Whether the "organisms" are ultramicroscopic or whether the hyaline bodies are the "organisms" is impossible to say at the present stage of our knowledge.

The spheres are found in large numbers in the very chlorotic regions of mosaic tomato leaves. The greater part of the spheres in these areas are without any trace of the chloroplast. In the non-chlorotic parts of mosaic leaves, the spheres, if present, usually have a considerable amount of the chloroplast mass adhering to them. But as a rule they are not so abundant in these regions as in the chlorotic areas. As to the presence or absence of the spheres in absolutely healthy plants, no definite results have been obtained. Special attempts to grow absolutely healthy plants were not made. On apparently healthy plants taken from the field, several hundreds of observations were made. In the majority of cases the chloroplasts were normal and did not have any spheres. Sometimes one or several spheres were found in the whole mount. This condition could not be compared with the abundance of spheres in the mounts from definitely diseased material.

In order to find out whether the spheres are found in insects, smear preparations of the contents of the intestines were made from white fly and mealy bug which were feeding on mosaic tomato. The methods of fixation and staining were the same as those used for the spheres in the plants. Photomicrographs (Pl. XV, E, F) show spheres from the insects to be indistinguishable from those found in the plant.⁴

“MOSAIC BODIES” OF TOMATO LEAF COMPARED WITH SOME
OF THE INCLUSIONS FOUND IN CELLS OF ANIMALS
AFFECTED WITH VIRUS DISEASES

Of the different pathological cell inclusions which are found in various animal virus diseases, the polyhedral bodies of lepidopterous larvae deserve special attention. They were studied by Prowazek (27, 28, 29), Glaser and Chapman (8), Komarek and Breindl (13), and others. The physico-chemical properties of these structures are well known. The polyhedral bodies from *Lymmonacha*, according to Komarek and Breindl (13), are 5–10 μ in diameter and tetrahedral in shape with somewhat rounded angles. In the young stage they are spherical. Their structure is homogenous. They are transparent and reflecting, and contain rapidly moving bodies. They give positive protein reaction, are not soluble in alcohol, chloroform, ether, or benzol, are comparatively resistant to weak acids, and are very sensitive towards alkali. When stained with Löffler's fuchsin red, they show definite structure; after Giemsa stain they appear blue, the peripheral part always being deeper in color.

The writer did not have opportunity to see the preparations of the polyhedral bodies, but, from the drawings as represented in the above mentioned papers, they resemble somewhat the spheres from diseased tomato. The size of the two structures is the same, and some of the physical and chemical properties, as well as their staining reactions, correspond very closely. Nevertheless, very essential differences should be emphasized. In the spheres, the positive protein reaction was absent. According to Prowazek (28), however, the protein reaction in the polyhedral bodies is not always positive. A very interesting analogy could be found between the polyhedral bodies and the spheres. In the diseased nuclei of the insects the chromatin material flows together and the polyhedral bodies arise as very minute structures. They gradually increase in size, and the chromatin is used up during the synthetic process. As the polyhedral bodies grow, they become more and more refractive, and stain with difficulty. Finally, the whole nucleus is transformed into a mass of polyhedral bodies. Similarly, in the

⁴ The difference in size of the photomicrographs is due to the difference in magnification.

chloroplasts of the diseased leaves the spheres are formed as a result of breaking down of the body of the chloroplast.

As to whether the polyhedral bodies are the degeneration products of the host, or a certain stage in the development of a parasitic organism, opinions differ. Knoche [according to Komarek and Breindl (13)] finds that they are a resting stage of an unknown organism. Glaser and Chapman (8) and Prowazek (27, 28, 29) think that they are products of the host. Komarek and Breindl (13) concluded that the polyhedrons are not real cysts or spores of an organism but are specific products of reaction of the nuclear substance, which could be compared with the formation of certain galls. But at the same time, the polyhedrons are the final stage in the life history of a Chlamydozoon, representing a resting stage of the latter.

In the cells of tomato leaf affected with mosaic, which were devoid of the normal components, there were hyaline bodies which moved with great rapidity. Some of these bodies were almost identical with the bodies found in the spheres. These bodies were very sensitive to alkali and resistant to many other chemicals. In addition to the bodies identical with these found in the spheres, there were also moving bodies of slightly different type. These were mostly much larger, between 2 and 5 μ , more irregular in shape and more sensitive to some chemicals. They reminded one of pictures of the hereditarily transmitted microorganisms of the class of Rickettsia, which were found by Cowdry (2) in some insects, and apparently unassociated with any disease. An interesting phenomenon was described by Cowdry (3) in the heartwater disease of cattle, which is due to virus transmitted by the bont tick. He found that in the adults, larvae, and nymphs of *Amblyomma hebraeum*, side by side with *Rickettsia ruminantium* which has been proved to be the etiological agent, there were other organisms apparently unassociated with the disease. They are larger than most of the Rickettsiae, decidedly pleomorphic, gram-negative and intracellular. In some cases the entire microorganism appeared brightly luminous, while in others the luminous material was confined to a thin marginal layer. The organisms varied from spherules to straight and curved rods and filaments. Similar microorganisms transmitted hereditarily were present in at least sixteen species of ticks.

Hertig and Wolbach (10) stated that minute microorganisms morphologically and tinctorially similar to the pathogenic Rickettsiae are widely distributed in arthropods, without relation to their feeding habits.

As mentioned above, at the present stage of our knowledge we cannot say whether the hyaline bodies are organisms or not. In some of their properties they correspond with our conception of an organism; in others they do not. The wide distribution of various kinds of hyaline bodies in plants is not negative evidence of the relation of some of these structures

to the mosaic disease. The literature we have reviewed on the organisms of similar structure in insects suggests that it is quite possible they are parasitic as well as symbiotic. This would explain the occurrence of symbiotic Rickettsiae in insects which are feeding exclusively on plant juices.

A third type of moving body was found in abundance in the hair cells. These bodies are very similar in structure to the hyaline bodies found in the spheres, except that they are not green reflecting. In all other properties they correspond exactly with the hyaline bodies in the spheres. Plate XIII, F is a camera lucida drawing of a living hair cell containing the bodies. They occupy the central part of the cell. There is very intensive, typical movement. The components of the cell nucleus, cytoplasm and leucoplasts are shown to be normally developed. Plate XIII, G illustrates a peculiar transitional stage of the bodies from the motile into resting condition. After a period of observation, the whole mass of the bodies became suddenly surrounded by a halo of clear cytoplasm. The clump was distinctly heterogeneous, composed of the bodies and a matrix. All motility stopped as soon as the bodies were completely surrounded. Similarly, a tendency of the Rickettsiae to aggregate into clumps in the tissues and in the cells of mammals and insects has been described by Cowdry (3).

In Plate XIII, H is represented a cell which has been plasmolyzed by a sugar solution. Here the cytoplasm plasmolyzes quite independently of the bodies. The latter collapse together and surround themselves by a sort of membrane. In the fixed and stained material the bodies do not retain their typical shape. This probably is the reason that very little attention has been paid to them. Possibly, however, Iwanowski (12) had in mind this kind of "organism" when he mentioned the occurrence of bacteria in the cells of tobacco leaves affected with mosaic. And the granular substance reported by several authors quite probably is a mass of fixed moving bodies.

In order to be sure of the effect of fixation on these bodies, the whole process of killing and staining was performed on the slide under the microscope, so that each transformation of the moving bodies was observed. In Plate XV, H is represented a hair cell from a mosaic tomato leaf fixed with Zenker's fluid and stained with gentian violet and eosin. The cytoplasm in this cell occupies the center; it is plasmolyzed and stained pink, and the nucleus is violet. Between the cytoplasm and the cell wall the bodies are found in abundance. They are brilliant violet-blue. Figure G shows the bodies localized only in one part of the cell, the cytoplasm having been destroyed in this part. The same combination of stains already described shows that the area occupied by the bodies is colorless, and the bodies stain a deep blue-violet, while the rest of the cytoplasm of the hair cell stains pink. The effect of the bodies on the stain is shown in Plate XV, I. The cell to the left is more or less healthy, it stains pink, and does not show any abnor-

mal inclusions. The cell to the right stains blue-violet on account of the large number of bodies inside it.

Strongyloplasma iwanowskii was the name given by Palm (26) to the supposed causal organism of mosaic disease in tobacco. He found some minute granules in the diseased cells, which he believes correspond to the organisms described as bacteria by Iwanowski. Some larger granules were recorded also. Apparently the granules of two different sizes noted by Palm correspond to the two types of bodies found in tomato mosaic.

The question whether the moving bodies are real organisms or the by-product of the activity of an ultramicroscopic organism, as has been mentioned, is very difficult to answer. One thing, however, is certain. An action of an organism is involved in the processes described and this organism possesses unusual properties. Some of these properties correspond very closely with certain properties of Chlamydozoa, *Strongyloplasma* (21), and *Rickettsiae*, which are already accepted by the protozoologists as being real organisms. Therefore it seems quite logical to conclude that an organism of the Chlamydozoon type is involved in the mosaic disease of tomato, the term Chlamydozoa being used here for a collective conception.

The fact that the destruction of the chloroplasts in tomato mosaic is associated with a Chlamydozoon type of organism is of great importance, because it provides a link between this type of disease and the animal virus diseases, the greater part of which, it has been demonstrated, are caused by an organism of this kind.

Kunkel (15, 16) thinks that the intracellular bodies found in plants with mosaic disease call to mind the intracellular bodies associated with certain of the virus diseases of animals. But in the animal diseases there are always present some minute inclusions of the chlamydozoa type inside of the bigger intracellular bodies, as, for instance, in the Negri bodies. The inclusions are really considered as the organisms which cause the disease. Such inclusions it seems are absent from the intracellular bodies of the mosaic-affected plants. Magrou (22) finds, on the other hand, that some of the pictures of Kunkel and the descriptions of Palm are very similar to a *Rickettsia* type of organism.

Among the other inclusions in cells of tomato affected with mosaic may be mentioned intracellular bodies of Iwanowski (12), Kunkel (15, 16, 17), Palm (26), McKinney, Eckerson and Webb (23), Goldstein (9), Smith (32), Cook (1), Rawlins and Johnson (30), and others. These bodies are quite definitely associated with the disease and are always found in the cells of mosaic tomato in which there are many crystals. The physical and chemical properties of these intracellular bodies correspond exactly with the properties of the living protoplasm. They are immediately killed with all of the reagents which ordinarily kill the cytoplasm of most cells, and do not

correspond with the peculiar reaction of the virus to certain chemicals like alcohol, acetone, acids and so on. Possibly they represent one of the stages of the life history of an organism.

The presence of crystals is very typical of cells in which intracellular bodies are found. Very commonly it is possible to see in one and the same cell intracellular bodies and the two types of moving hyaline bodies.

As to the "striate material" described by several authors, such a formation has never been observed in living cells. In the fixed material it is a quite constant inclusion of the cells which contain the intracellular bodies. By fixing the material under the microscope it was possible to observe the transformation of the crystals into striate material. Especially effective in this respect is picric acid. After half an hour in one per cent water solution, all of the large crystals were transformed into striate material.

SUMMARY

1. In the decidedly chlorotic region of a young leaf of tomato affected with severe mosaic disease, all of the cells are nearly isodiametric. There is no differentiation of tissue into palisade and spongy parenchyma. In a majority of the cells the chloroplasts are absent or present in small numbers. A granular substance often occupies the cells in which the normal components are lacking.

2. The chloroplasts are destroyed through the dissolution of the proteins of the stroma. This fact is easily demonstrated by direct observation of the living material and by microchemical tests. The same is confirmed by study of the material fixed under the microscope, or fixed and stained by usual cytological methods.

3. The normal chloroplasts are rather solid. The first indication of a pathological condition is the appearance of rapidly moving hyaline bodies within the chloroplasts. The movement of these bodies is possible only after liquification of the stroma of the chloroplasts has taken place. Therefore it is assumed that a proteolytic enzyme, possibly secreted by an organism, is present. An increased osmotic concentration results inside of the chloroplast, and water is taken in from the surrounding medium. Finally, the entire body of the chloroplast goes into solution and, if sufficient water is present in the surrounding medium, spherical transparent vesicles result.

4. The sphere represents a "membrane" resulting from the digested chloroplast in which the digestion products and water are retained, the whole being surrounded by a surface film at the interface. The spheres give negative protein reactions, and are not soluble in alcohol, acetone, or acids. They are soluble in weak alkali. They can be fixed and stained by the methods used in the study of the inclusion of the animal virus diseases.

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EXPLANATION OF PLATES

PLATE XII

Photomicrographs. Zeiss 8, Apert. 65, Comp. Oc. 8.

- A. Section through chlorotic region of a mosaic tomato leaf about three weeks old.
 - B. Section through a green area of the same leaf.
 - C. Section through the transitional zone between the two areas.
- All three photographs were from the same section.

PLATE XIII

Camera lucida drawings. Zeiss Apochr. Oil Imm. 3, Apert 1. 30, Comp. Oc. 12.

Reduced four times in reproduction.

- A. Surface view of a transitional zone of a mosaic tomato leaf, focused through the epidermis.
- B. Surface view of parenchyma cells from a chlorotic region of a mosaic tomato leaf. The transparent spheres result from the destroyed chloroplasts.
- C and D. Stages in the destruction of chloroplasts and formation of spheres.
 - 1.—Structure of a healthy chloroplast.
 - 2.—Early stage of disintegration of the chloroplasts showing stationary hyaline bodies surrounded by a halo.
 - 3.—Later stage of disintegration at which the hyaline bodies become motile.
 - 4.—A sphere appearing on one side of the chloroplast.
 - 5.—The sphere increased in size.
- D. 1.—The motile bodies in the sphere.
 - 2-5.—Gradual stages of the complete dissolution of the mass of the chloroplast.

- E. The hyaline bodies from the spheres. (Zeiss Apochr. 3, Apert. 1. 30, Comp. Oc. 18.) Some of the characteristic shapes of the bodies. From the front view the bodies appear more or less crystal-like with a definite black stripe connecting two black points. From the side view the body has a cap-shaped addition.
- F. Living hair cell from a mosaic tomato leaf. (Leitz 6, Oc. 10.) The development of the bodies in the cell vacuole. The bodies have a very distinct and rapidly rotating movement and are highly luminous.
- G. The same cell as in F. The bodies suddenly clump together, lose their motility, and assume a membrane.
- H. Hair cell, similar to one described in F, after plasmolysis. The cytoplasm plasmolyzes independently from the mass of the bodies, which in turn clump together.

PLATE XIV

Motion photomicrograph of living chloroplasts and spheres.

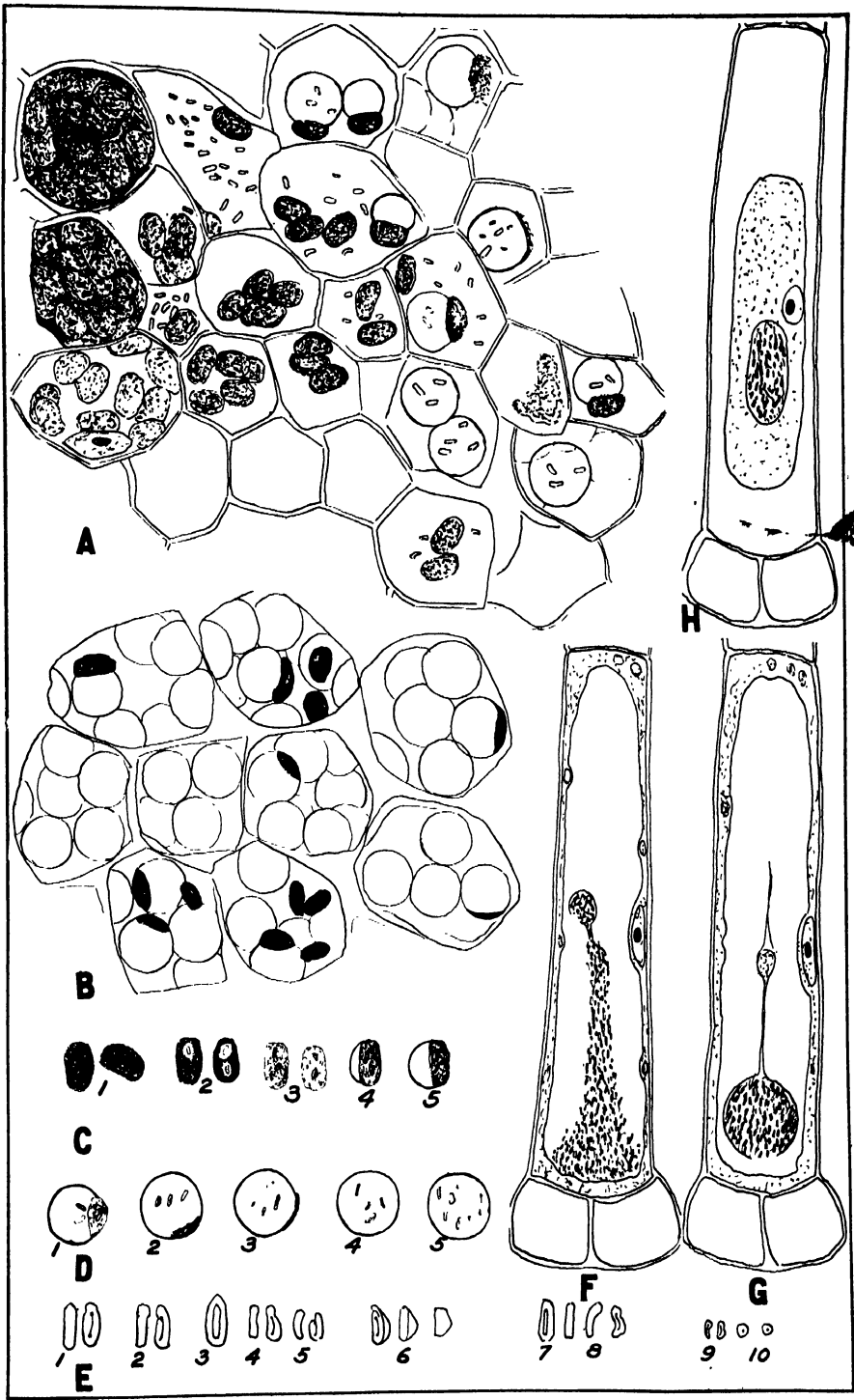
- A. A print from the positive. The white bodies are the chloroplasts. The transparent vesicles are the spheres. Different stages in the dissolution of the bodies of the chloroplasts are shown in the print.

PLATE XV

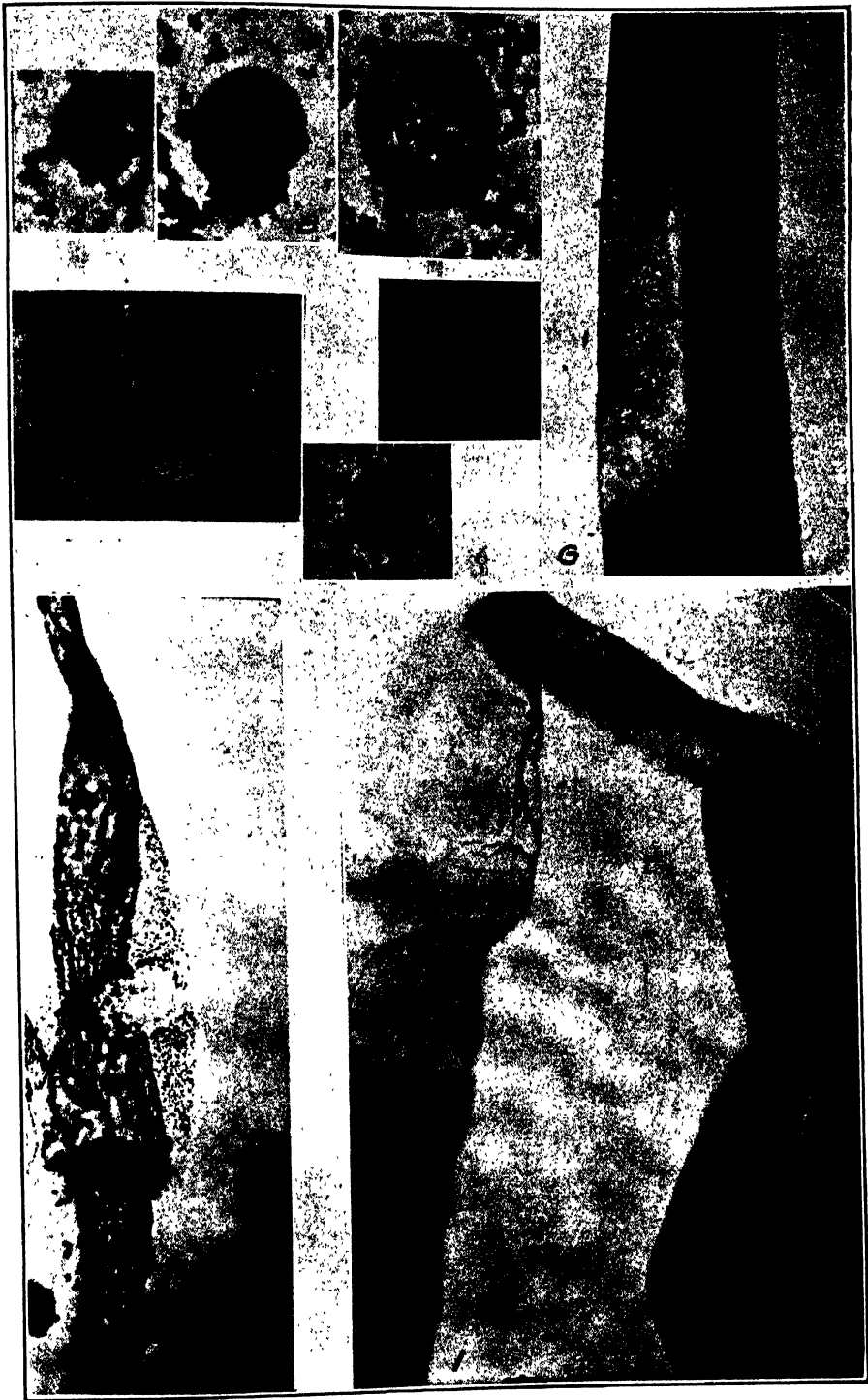
Photomicrographs. Zeiss Apochr. 3, Apert. 1. 30, Comp. Oc. 12.

- A. Chloroplast stained with Löffler's stain.
- B. Chloroplast in the stage of destruction. The dark body below is the chloroplast. The structure on top of it is the sphere. Definite granules and threads are visible inside of the sphere.
- C. Later stage in the dissolution of the chloroplast body.
- D. Series of stages similar to A, B and C, after Giemsa stain.
- E and F. Spheres from the intestines of insects feeding on diseased tomato.
- G. A hair cell from the diseased material fixed with Zenker and stained with gentian violet and eosin. The cytoplasm appears brilliant pink, the nucleus purple, the bodies, which occupy the space between the cytoplasm and the cell wall, are brilliant blue.
- II. A hair cell from diseased material treated as in G. The mass of the bodies is localized in a certain portion of a hair cell. The cytoplasm of the unaffected portion of the cell is pink, the bodies are brilliant blue. The portion of the cell around the mass of the bodies is white, the cytoplasm apparently being destroyed there.
- I. Two cells of the same hair, treated as in G. One to the left is healthy, the cytoplasm is homogenous in structure, and is stained pink. In the cell to the right there is an enormous number of bodies, so that the whole cell is stained blue.









SPECIES OF ASCOCHYTA PARASITIC ON THE PEA¹

MAURICE B. LINFORD AND RODERICK SPRAGUE

INTRODUCTION

Among the several diseases of the pea (*Pisum sativum* L.) which have demanded study as an outcome of the Wisconsin pea disease survey (9) is the well-known leafspot and podspot attributed to the fungus *Ascochyta pisi* Lib., the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone. Attention was drawn to this disease in 1924, when, in fields from which foliage spotting was absent, a footrot was found which yielded cultures of an *Ascochyta*. These cultures produced very small, chiefly non-septate pycnosporos and were regarded at the time as a *Phoma*-like fungus (9) but later (5) as a species of *Ascochyta*² probably distinct from *A. pisi*.

In 1925, when an attempt was made to compare this fungus with *Ascochyta pisi*, two distinctly different and markedly constant types of *Ascochyta* leafspot were observed in Wisconsin fields; one characteristically regular and light brown in color, the other irregular and dark brown. Measurements of pycnosporos revealed the presence of three distinct fungi associated with the two types of spots. The light-brown spots contain only a typical *Ascochyta* with spores larger than those of the footrot fungus. The dark spots, on the other hand, contain two different fungi as indicated by spore characters. The one usually present is a typical *Ascochyta* with spores somewhat larger than those found in light-brown spots. The other fungus, found less frequently, is apparently identical with the footrot fungus.

Likewise, isolations from these two classes of leafspots have yielded three different types of cultures. Cultures from the light spots fall readily into a group by themselves. Those from the dark spots are all similar in macroscopic appearance, but microscopically they are of two types with respect to spore characters. The predominant type is a typical *Ascochyta*, but less frequently the footrot fungus is found.

¹ Contribution from pea disease investigations conducted at Madison, Wisconsin, supported jointly by the Wisconsin Pea Packers' Association, The Wisconsin Agricultural Experiment Station, and the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture.

The writers wish to express their gratitude to Dr. F. R. Jones for his advice and assistance, both in the progress of this work and in preparation of the manuscript.

² This is probably identical with both Haenseler's (6) *Phoma* sp. and his "sterile fungus."

After Jones (10) had emphasized the importance of *Ascochyta* infection of pea seed for canning-crop production, numerous isolations of *Ascochyta* from seed were made at Madison, and the cultures thus obtained were found to fall readily into the three classes already recognized.

In this paper these three types of *Ascochyta*, isolated from two classes of leafspots, from footrot, and from diseased seed, are compared. For convenience, spots of the light-brown type, together with the fungus which they bear and cultures of the type isolated from such spots, are referred to as "light"; spots of the dark-brown, zonate class with the corresponding fungus and cultures are designated "dark"; and the footrot fungus and cultures are termed "micro." A partial report of this work has already been published (14).

SYMPTOMS ON AERIAL PARTS

The two classes of spotting caused by *Ascochyta* on pea leaves, stems, and pods, readily distinguished by observation of well developed symptoms, are as follows:

"Light"

The light type of spotting (Plate XVI, A-C) is characterized by a circular, tan spot, sometimes whitish at the center, with a distinct dark-brown margin, and with amber to brown pycnidia fairly conspicuous towards the center. Spots on leaves are circular or oval and from 2 to 10 mm. in diameter. On stems, petioles, and pedicels they are deep and oval or elongate, and may almost sever the affected part. On pods, deep, circular, tan pits bearing numerous pycnidia and surrounded by a prominent dark margin frequently penetrate and discolor the peas within. Late infection of older pods may lead to the production of pycnidia without the formation of definite spots.

"Dark"

At a glance this type of spotting differs from the light in its dark, irregularly dendritic or zonate character. Minute dark-brown points first appear, which may enlarge dendritically (Plate XVI, D), retaining a dark-brown, chocolate, or purplish-brown shade. On dried specimens these small spots often appear elevated. Becoming larger, they may assume a circular outline and a zonate aspect (Plate XVI, E-G). Zonate spots are usually lighter than the smaller, homogenous ones, but they do not approach the tan color of light spots. They are generally darkest at the center, sometimes pale-gray-brown in the peripheral zones. Frequently they are surrounded by a zone of affected tissue which is still green. Where spots are numerous usually but few attain a size as large as 8 mm. in diameter. On stems,

petioles, and pods this dark spotting is commonly more superficial and spreading than the light. Stem lesions occur especially near the ground and at nodes, and may spread over considerable areas, causing a dark-brown to purplish-brown discoloration of the cortical tissue.

Often abundant infection will result in only minute brown flecks and streaks. On pods such flecks occur frequently, but well developed spots are less common than with the light type. When they do occur, dark spots on pods are less abruptly sunken, darker, and usually purplish-brown to gray-brown in color, and are often zonate, as on leaves. Late infections may, however, lead to fruiting on the pod without forming any spots, a condition macroscopically indistinguishable from the similar development of the light fungus.

Pycnidia are produced erratically in spots of the dark type. Many spots, apparently well developed, contain none; and, when present, the pycnidia are inconspicuous against their dark background, and are scattered near the periphery rather than clustered at the center of the spot. Occasionally, however, they are abundant.

THE FUNGI

Morphological Differences

For a comparison of the fungi associated with these two types of leaf-spot, exsiccatae from various parts of the United States and Europe were examined. From these sources and from freshly collected specimens, 20 samples of each type of spot were selected, in addition to four samples of the micro fungus from freshly collected dark-type spots. Twenty-five spores from each sample were measured under the oil immersion lens.

Considerable variation was observed between the various samples of each class, spores from podspots being more variable than those from leafspots; but the spores from light spots are clearly shorter and somewhat narrower than the spores from the dark ones, excepting only that the micro spores from occasional dark spots are smaller still. Mean spore lengths of light samples are nearly all less than 13μ , while those of the dark are, with two exceptions, greater than 13.5μ (Fig. 1). Differences in breadth are evident but less striking.

Visual examination of the spores reveals certain differences not brought out by measurements. The dark spores appear plumper and more constricted than the light, and fresh spores of the former contain more conspicuous oil droplets. Counts of the spores of the micro fungus show that 35 to 72 per cent are nonseptate, and that the remainder are uniseptate or very rarely biseptate. Both light and dark spores are practically all septate at maturity, rarely forming even three septa (see Plate XVII, A-C).

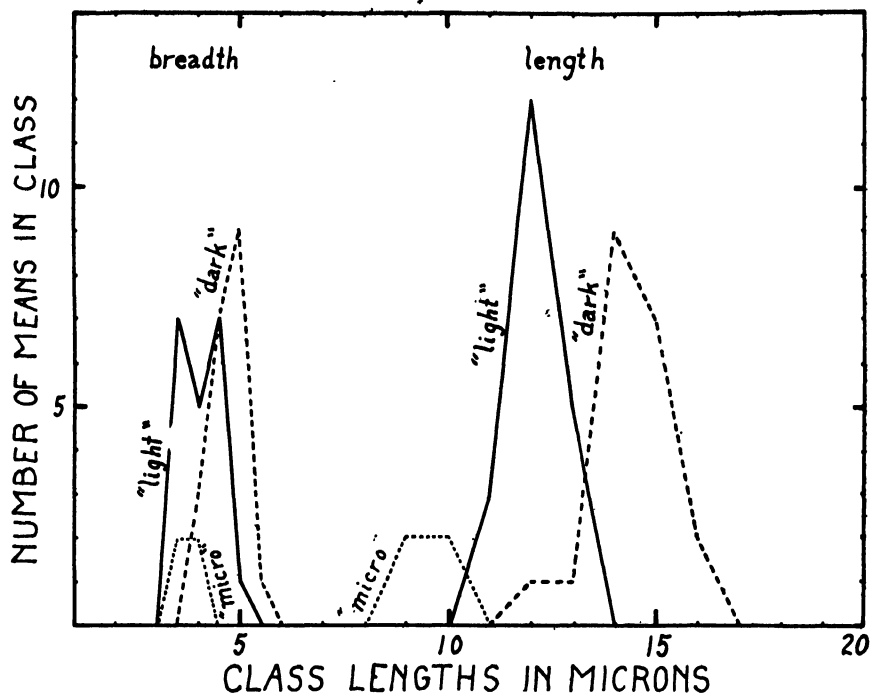


FIG. 1. Distribution of mean breadths and mean lengths of 20 samples of 25 spores each of "light" and of "dark" species, and of 4 samples of 25 spores each of the "micro" *Ascochyta*.

*Comparison in Culture*³

"Light"

Growth is moderate on slants and plates, producing a compact, fluffy, white or very lightly tinted colony, coloring the agar a rich amber or brown. Pycnidia are not produced immediately except in contact with the walls of the culture vessel, but in tubes after 10 days or more, as the agar shrinks away from the glass, a thin mycelial growth covers the new surface and pycnidia usually form in abundance. The pycnidia are brown and they exude spore masses of a clear pink color. With age the mycelium may become matted, and generally assumes the buff color mentioned by several workers. In petri dishes the colonies seldom exceed 7 centimeters in diameter.

Pycnospores are almost cylindrical, slightly constricted, and with rounded ends. They are slender for their length: $11-14 \times 3-4 \mu$; length-breadth ratio 3.7-3.5 (Plate XVII, D).

³ These descriptions are based on single pycnospore-cultures grown on potato-dextrose agar except when otherwise specified: 200 g. potato, 20 g. dextrose, 17.5-20 g. agar, in 1000 cc. water.

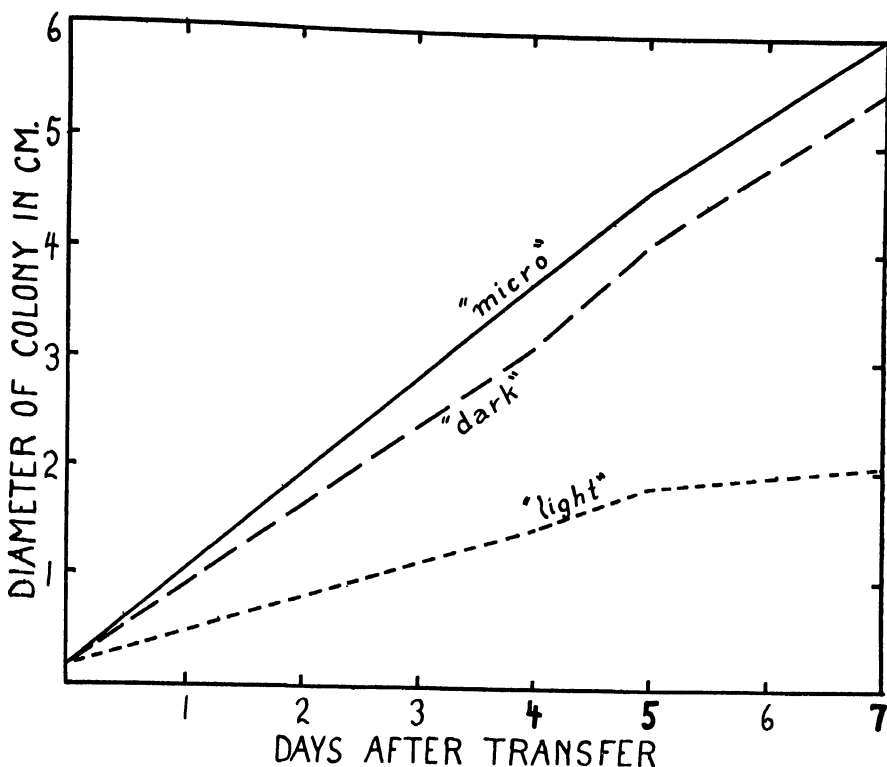


FIG. 2. Average rate of colony growth of the three types of *Ascochyta* on potato dextrose agar in petri dishes at 24° C. Measurements graphed are averages of measurements of 32 isolations of "dark," 16 of "micro," and 4 of "light" fungi.

"Dark"

Growth is more rapid on slants and plates, forming a flat colony at first whitish or light-grey, soon becoming dark olivaceous to black at the center. Pycnidia are produced abundantly after four days at 23° C., arising either from shining, black, radiating strands or scattered in the light mycelium. Pink spore masses are exuded copiously. After this sporulation, a more fluffy olivaceous or sometimes lighter growth spreads over the colony. A few pycnidia are often produced in this fluffy growth, generally surrounded by a mass of dark mycelium, giving old slant cultures a mottled appearance. On plates the dark cultures increase in diameter more than twice as fast as the light, as shown in figure 2. Pigmentation of the potato dextrose agar is almost wholly lacking; or, when developed, blackish but never rich brown.

Pycnospores are relatively plump and distinctly constricted, containing conspicuous oil globules. They measure about $10-13 \times 4-5 \mu$; length-breadth ratio 2.5-2.6 (Plate XVII, E).

“Micro”

This differs from the dark in slightly faster growth (Fig. 2), less aerial mycelium, darker color with more glossy black and olivaceous growth, and less tinted spore masses, but, without microscopic examination of the spores, separation of micro from dark cultures is impossible (Plate XVII, F). The spores, which are usually nonseptate, measure $5-9 \times 2.5-4 \mu$ on potato dextrose agar, and $3-6 \times 3-4 \mu$ on oatmeal agar. Considerable numbers of uniseptate spores are produced on some media (steamed pea seed). Amount of fruiting is more variable than with light or dark cultures, some isolations from known micro leafspots producing no pycnosporos in culture.

Several cultures of the micro fungus, particularly the blacker cultures which produce few pycnidia, develop chlamydospores in great profusion and variety (Plate XVII, G). They may be small, citronshaped, continuous or uniseptate, olivaceous spores formed from the segmentation of irregular or zigzag hyphae, or they may be large, fusiform, oval, or spherical structures of one to three cells, dark-brown in color and filled with large oil droplets. These large spores are borne sometimes in chains, sometimes apically, and are very striking in their appearance. The smaller spores occur in most cultures of the micro type and have been seen in some typical dark cultures.

Crystalline Deposits in Agar

Deposits of two distinct types, apparently crystalline in nature, occur frequently in potato dextrose agar beneath colonies of all three classes of *Ascochyta*. In the substrata of dark and micro cultures there appear compound fan-shaped structures composed of minute, slender, white needles, and resembling strikingly the frost figures formed on window-panes on winter nights. In cultures of the light group, minute golden-yellow specks are formed as far as 8 millimeters below the surface of the agar. Microscopically they appear as spherical asters of fine radiating filaments, some flexuous but others rigid and bent repeatedly at right angles. The dense yellow centers of these asters are generally not in direct contact with the immersed hyphae. Such deposits, though their nature has not been determined, constitute evidence of a close physiological relationship between dark and micro and a distinct separation of these two from the light *Ascochyta*.

Variability and Saltation

In dealing with large numbers of isolations of the three types of *Ascochyta* during a period of 12 months for light and dark and 28 months for micro cultures, variability has been noted especially in both dark and micro cultures, but none of the variants have been seen to approach closely the more nearly constant light type of culture. The dark and micro forms do,

however, show similar types of variation in degree of fluffiness, in color, freedom of fruiting, and rate of growth. Both show the phenomenon of saltation as noted in different fungi by Leonian (12) and others. A single-spore colony may split into sectors of very different appearance and habit of growth, and preliminary work indicates that some of these segregates remain constant through a number of mycelial transfers. These saltations have not yet been seen to involve pyrenospore characters, but whether the micro fungus is a well fixed form or merely a frequently recurring saltant or mutant from the dark awaits determination.

INOCULATION EXPERIMENTS

To determine the relative pathogenicity of these three groups of cultures, several inoculation experiments have been conducted on both aerial and subterranean plant parts.

Aerial Parts

In a foliage inoculation experiment begun October 29, 1925, potted peas of Alaska and Horsford's Market Garden varieties were sprayed with pyrenospore suspensions from one single-spore culture each of light, dark, and micro types. On December 4, the light fungus had produced typical light spotting; the dark fungus had produced abundant dark-reddish-brown lesions on leaves and stems, some of them zonate and with a few pycnidia; and the micro fungus had formed a few spots similar in appearance to those produced by the dark fungus but without pycnidia. Symptoms were the same on both varieties of peas. Micro spots, when placed in a moist chamber, formed pycnidia with typical small spores. Reisolations yielded, in all cases, cultures indistinguishable from those used for the inoculation.

A much more extensive series was run in August, 1926, using 38 single-spore cultures of the three types obtained from leafspots, footrot, and pea seed. Potted Yellow Admiral peas in the fourth node stage were sprayed with spore suspensions prepared from cultures on potato dextrose agar. One pot of peas was inoculated with each culture, and six pots were retained as uninoculated controls. All were then placed in a spray chamber, where they were held at a temperature of 22–23.5° C. for 64 hours, and then removed to the greenhouse for one day, and returned to the spray chamber at night. They were then removed permanently to the open greenhouse.

The first infection was noted on plants inoculated with dark and micro fungi at the end of 65 hours, when minute, dark-brown flecks were seen on leaves and stems. Inoculation with the light cultures showed no infection until the fifth day, when a few collapsed areas, still green, were noted. On the ninth day, 10 of the 17 pots of peas inoculated with light cultures showed typical stem and leaf lesions, many of them with conspicuous

pycnidia. At this time practically every plant in the 14 pots inoculated with dark cultures and the seven pots inoculated with micro cultures was spotted with numerous points of infection. These infections developed farther, finally showing a complete range of flecks, dendritic spots, zonate spots, and irregular blackening of stems, as observed in the field, all distinctly darker than the definite spots of light type. Dark and micro spotting were indistinguishable. Reisolation yielded cultures of the three fungi indistinguishable from those used for inoculation. The uninoculated controls remained free from spotting throughout. *

These experiments show a constancy of correlation between fungus type in culture and type of spotting on the host, and a lack of intermediate symptoms between light and dark, but with micro and dark symptoms indistinguishable. The types of spotting were not influenced appreciably by the three different varieties of peas used in these two experiments.

Footrot Inoculations

Earlier work has indicated that both *Ascochyta pisi* Lib., as previously known, and the micro fungus may cause footrot similar to the *Fusarium* stem and rootrot of peas caused by *F. martii* App. and Wr., var. *psii* F. R. Jones, but to compare the pathogenicity of the three fungi here considered the following experiments have been conducted.

In the first experiment, Alaska peas were grown in steamed soil in metal cans held in the Wisconsin soil temperature tanks at soil temperatures of 12°, 20° and 28° C. They were inoculated when planted with crushed cultures of the three fungi grown on steamed pea seeds, one culture tube containing 15 peas per pot. Twenty-eight days from planting date (Oct. 24, 1925) the roots were washed and examined. The light fungus gave no infection at the two lower temperatures, but at 28° C. seven plants or 30.4 per cent were affected with a superficial, yellowish softrot, from which only *Pythium* sp. and bacteria were isolated. The dark and micro fungi both produced well developed footrot symptoms, with the micro evidently causing the more severe injury as well as infecting a larger percentage of plants. The temperature factor appeared unimportant.

A second more extensive experiment was undertaken with 43 different cultures of the three fungi, but owing to the fact that the seed used apparently carried some *Ascochyta*, it need not be reported in detail. Of the plants inoculated with light cultures, 7.6 per cent developed typical footrot, which was less than the 9.1 per cent of footrot in the controls. Dark inoculations gave 63 per cent, and micro gave 87 per cent footrot, both sufficiently higher than the controls to establish the aggressiveness of these two as parasites. The light fungus is, then, clearly less pathogenic if not impotent as a cause of footrot, while both dark and micro fungi may be aggressive in

their parasitism at the base of the epicotyl and top of the taproot of the pea plant.

TAXONOMY

From the foregoing it is apparent that three fungi belonging to the genus *Ascochyta* Lib. are of common occurrence as parasites upon *Pisum sativum*. Two of these, designated dark and micro, have many characteristics in common, while the third, the light, is so widely different from them both on the host and in pure culture that it merits specific rank by itself. In the past, at least two of these, dark and light, have been listed in both literature and exsiccatae under the name of *Ascochyta pisi*.

This name was given in 1830 to a collection on pods of *Pisum sativum* from France, distributed by Madame Libert (13) with a brief description on the packets. This description, and also symptoms and spore measurements ($11-15 \times 3-4 \mu$; mean, $12.7 \times 3.5 \mu$) of a packet of Libert's collection in the Farlow Herbarium at Harvard University identify this with our light fungus.

Descriptions of presumably the same fungus were published shortly afterward by Link, Berkeley, and others. Link's (15) description is brief and follows Libert's closely. Berkeley (1) described spots on both pods and leaves which are certainly of the light type under the name of *Sphaeria* (*Depazea*) *concava* Berk., but in 1860 (2) he listed this as a synonym of *Ascochyta pisi*. Meantime, in 1859, he had published *Sphaeria* (*Depazea*) *pisicola* Berk. (3) as a new species with a brief description including the spore length of 0.0004-0.0005 inch ($10-13 \mu$), which identifies this also with our light fungus. What distinction Berkeley saw between his two species is not revealed in his descriptions. *Ascochyta pisicola* (Berk.) Sacc. is thus a synonym of *A. pisi* Lib. as suggested by Stone (19), and our light fungus is this species.

Descriptions of *Ascochyta pisi* usually given, as those of Saccardo (17), and Davis (4), are based upon the light type of spotting, this apparently being regarded as the typical development of the species, but the spore measurements most commonly given ($14-16 \times 4-6 \mu$) are larger than the largest sample we have obtained from a light spot and agree more closely with our measurements of the dark fungus.

Both light and dark fungi were present in Europe as early as 1895, for the fungus described by Krüger (11), and Hiltner (8) as the cause of serious footrot and seedling blight was clearly the dark species, and both are present in a packet of Krieger's Fungi Saxonici 1548 collected in 1895. In America, Van Hook's (20) illustrations, descriptions, and spore measurements indicate that he met with both types, and Stone (19, p. 565) described both without differentiating.

Krüger (11) first suspected what was later considered established by Stone (19) and Vaughan (21), that *Ascochyta pisi* as then understood is a pycnidial stage of *Mycosphaerella pinodes*. This work appears to have been done, however, with our dark species and not the true *A. pisi*.

Krüger was dealing with a virulent footrot parasite which turned the vines black. Stone's paper indicates that it was the dark fungus with which he was chiefly concerned, and Vaughan has personally identified our dark fungus as his *Mycosphaerella pinodes*. In addition to these lines of evidence we have found perithecia with mature ascospores of this *Mycosphaerella* in some dark cultures on Melilotus stems, and single ascosporous cultures from these have produced only the dark culture. The dark fungus and probably not *A. pisi* is thus the pycnidial stage of *M. pinodes*.⁴

With our dark fungus thus placed as the imperfect stage of *Mycosphaerella pinodes*, it requires description⁵ to distinguish it from *Ascochyta pisi*. This latter fungus, now left unattached to a perfect stage, regains its former designation, but the description requires slight emendation. The micro fungus will be described tentatively for convenience without attempting to give it final taxonomic treatment.

Ascochyta pisi Lib.

Spots definite, circular on leaves and pods, elongate on stems, yellowish-brown with dark-brown margin often prominent, sometimes whitish in center, spots not defined on mature plant parts; pycnidia gregarious at center or in circular zones, somewhat prominent, depressed-globose, light to dark-brown, ostiolate, 75–225 μ ; spores hyaline, oblong, ends rounded, straight or somewhat curved, 1–(1–3) septate, somewhat constricted, 10–14 (8–19) \times 3–5 (2.5–6) μ , mean length less than 13.5 μ .

On leaves, stems, pods and seeds of *Pisum sativum* L.

Mycosphaerella pinodes (Berk. and Blox.) Stone.

Pycnidial Stage.—Spots irregular to circular-zonate, not definitely margined, dark-brown, sometimes lighter when zonate, spots not defined on mature plant parts; pycnidia scattered, rarely at center, somewhat prominent, irregularly depressed globose, light to dark-brown, indistinct in spot, ostiolate, 65–175 μ ; spores hyaline, oblong, ends rounded, straight or somewhat curved, 1–(1–3) septate, sharply constricted, 12–17 (10–21) \times 3.5–5.5 (3–7) μ , mean length more than 13 μ .

⁴ The following abstract presenting evidence in essential agreement with ours that *A. pisi* and *M. pinodes* are two separate organisms appeared after this paper had been submitted for publication.

Jones, L. K. The relation of *Mycosphaerella pinodes* to *Ascochyta* blight of peas (Abst.). Phytopath. 17: 44. 1927.

⁵ The description given by Stone (19, p. 581) is too general to serve diagnostically.

On leaves, stems, pods, and seeds of *Pisum sativum* L. Also causes firm, dark-brown rot of epicotyl and upper taproot.

Mycosphaerella pinodes (Berk. and Blox.) Stone, micro form?

Similar to pycnidial stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone; spores continuous to 1-septate, continuous spores oval to oblong, $5-11 \times 2.5-4.5 \mu$, uniseptate spores oblong, rounded at ends, more or less constricted at the septum, $8-12 \times 3-4.5 \mu$.

On leaves, stems, pods, and seeds of *Pisum sativum* L. in Wisconsin. Also causes firm, dark-brown rot of epicotyl and upper taproot.

This revision of the old conception of the species *Ascochyta pisi* throws into uncertainty work done hitherto on the host range of this species. Scalia's (18) attempt to mass the legume-infesting species of *Ascochyta* under this name, upon morphological bases, seems now especially in need of review. Very little can yet be said as to the identity of the species of *Ascochyta* reported on a considerable range of species of *Vicia*, *Lathyrus*, etc. In Wisconsin, an *Ascochyta* spot, referred to *A. pisi*, is of common occurrence on *Vicia angustifolia*, and the type of spotting is rather similar to the authentic *A. pisi* on peas. Further, the fungus isolated from such spots from three separate localities agrees in bearing very close resemblance in general appearance to *A. pisi* and in being totally unlike our cultures of *Mycosphaerella pinodes*. No detailed study of the fungus on vetch has been made. Further study should attempt to find the perfect stages of *A. pisi* and of the micro fungus, if any, and to determine the range of variability and parasitism of these three fungi.

SEED INFECTION

It is known from the work of Halstead (7), Krüger (11), Hiltner (8), Van Hook (20), and others that *Ascochyta pisi*, as formerly understood, is carried freely on pea seed. In the present study numerous samples of seed have been tested⁶ to determine which of the three fungi here distinguished are thus carried.

Transfers to slants, from 94 samples of Wisconsin and Eastern grown seed thus tested, included all three of these *Ascochyta* forms and some other fungi. Twenty-eight samples yielded *A. pisi*; 23, *M. pinodes*; 5, the micro fungus (9 samples contained two or three of these fungi); and 8 other infested samples were not classified. Not only was *A. pisi* most frequently

⁶ A sample of peas (50 or 100) was immersed 30 seconds in 70 per cent alcohol, transferred to 0.1 per cent $HgCl_2$ for 1 minute, and washed for 2 hours in three changes of sterile water. The peas were then drained and planted, six to eight in a petri dish, pressed down into a deep layer of 1 per cent agar, acidified with lactic acid. Potato dextrose was generally used, but Dox's medium was used with success in some instances.

found in the samples, but these samples also contained a somewhat higher average percentage of infected seeds than the samples in which *M. pinodes* or the micro fungus was found. This is significant in view of its negligible importance as a cause of footrot. The total of 54 infested samples showed an average of 8.4 per cent of seeds affected with these three fungi; the highest percentage in one sample was 33, but several tested 20 per cent or more. These fungi occurred in samples from widely separated localities in Wisconsin and, collectively, in the following varieties of peas: Alaska, Winner, Yellow Admiral, Green Admiral, Advancer, Perfection, Horsford, Ashford, Horal, Badger, Rice's 13, Prince Edward, and some unnamed selections.

PLOT TESTS

From the above seed tests, 24 samples of infested seed, together with 7 samples of *Ascochyta*-free Wisconsin and eastern-grown seed and 19 samples of clean Idaho-grown seed representing several varieties, were selected for a plot trial to learn something of the significance of seed-borne species of *Ascochyta*. Two hundred seeds of each of these samples were planted in duplicate in rows 3 feet apart, the seeds about 1 inch apart in the row. Planting was begun May 14, somewhat later than the middle of the planting season for canning crop peas. Rains forced a delay in planting, and the remaining peas were planted May 20-22 while the surface soil was wet and sticky. When the early varieties were beginning to bud, from June 18 to 22, one of the duplicate rows of each sample was dug, counted, and examined for footrot symptoms. The other row was left standing to maturity for a determination of *Ascochyta* spotting of the vines.

The plants, as dug, were notably clean, comparing favorably in appearance with what may generally be noted in late sown commercial fields. The rows planted from badly infested samples appeared, in general, as clean as the controls, all of them bearing more or less frequent lesions caused by *Rhizoctonia*. To check upon minor differences, calculations were made of percentage of stand, percentage of plants infected, percentage of plants cut off below ground and sprouted again, and infection-rating, as summarized in table 1, but no significant correlation with infested seed was found except in the case of percentage of stand. Infested samples as a group gave poorer stands by a percentage figure which agreed closely with the average percentage of infected seeds in the samples, but individual samples fluctuated widely from this average correlation. No significant differences between *A. pisi*, *M. pinodes*, or mixtures of these with the micro fungus could be detected. Since germination tests of the seed used in this plot work were not made, this reduction of stand noted in infested samples cannot be analyzed precisely.

The other interesting result of this trial was that the early planting gave better stands than the late; the infested samples in the early planting did as well as the controls planted one week later under the different environmental conditions then prevailing.

Aside from these differences in stand, the infested samples were practically indistinguishable from the controls, showing, if anything, slightly lower percentages of footrot and lower infection ratings. Obviously there was no significant influence of the seed-borne fungi in this one season trial other than possibly that of reduced stand.

The duplicate rows in this trial plot came to a normal maturity and developed only tardily a slight trace of leaf spotting.

TABLE 1.—Summary of data from plot test of seed infested with *Ascochyta* species compared with *Ascochyta*-free seed

Planting dates	Seed samples		No. of rows of 200 seeds	Seeds infected per cent	Stand per cent	Plants infected per cent	Footrot infection rating ^a
May 14, 1926	Controls, Idaho grown		9	...	88.5	36	7.8
	Infested	<i>A. pisi</i>	4	13	78.8	19	3.7
		<i>M. pinodes</i>	3	3.3	86.6	25	3.6
		Mixed	2	7.5	71.7	24	5.4
		Undetermined	2	6.5	80.7	45	16
	Total infested samples		11	8.2	80	27	6.2
May 20-22, 1926	Controls	Idaho grown	10	...	79.4	29.3	7.7
		Wisc. grown	7	81.8	30	6.8
	Total controls		17	80.4	30	7.3
	Infested	<i>A. pisi</i>	2	11	47.2	29	7.8
		<i>M. pinodes</i>	2	8	79.7	38	6.1
		Mixed	6	12.3	70.3	24	3.7
		Undetermined	3	6	72.3	38	8.9
	Total infested samples		13	10	68.6	30	5.9

^a Method of calculation modified from McKinney (16).

FIELD OBSERVATIONS

A continuation of the Wisconsin pea disease survey in 1925 found *Ascochyta* spotting more prevalent than in 1924 (9), occurring in 9.4 per cent of the 700 fields surveyed. In several instances it clearly was seedborne. For example, one lot of Pedigreed Extra Early peas imported from a humid region was planted in two widely separated counties in Wisconsin. In both places every field planted from this seed developed the light *Ascochyta* leaf-spot, while no such spotting could be detected in fields planted from other

seed stocks in the same localities. Elsewhere in the State, similar spotting was seen in a number of lots of both canning and field varieties from Wisconsin seed, but none of these fields bearing the light *Ascochyta* (*A. pisi*) suffered any marked injury. The few instances of moderately severe injury from *Ascochyta* observed in the survey were in fields where peas were planted immediately following peas without rotation and where the dark *Ascochyta* leafspot (*Mycosphaerella pinodes*) was present. However, during the past three years in Wisconsin serious injury from *Ascochyta* species has been all but absent, and both bacterial blight (*Bacterium pisi* Sackett) and anthracnose (*Colletotrichum pisi* Pat.) have been more important foliage diseases.

Peas grown for canning in several localities in Wisconsin from seed tested and found to contain one or both species of *Ascochyta* were observed through the season of 1926. A lot of Perfection peas which tested 24 per cent *Ascochyta*, both *A. pisi* and *M. pinodes*, developed small and varied amounts of dark *Ascochyta* spotting on leaves and stems in five fields observed, as well as footrot (6, 29, 13 and 8 per cent) in four of them. A few plants were markedly weakened, but yields were excellent and quality of peas very good. In the same locality two fields planted from clean Idaho grown Perfections showed 17 and 27 per cent footrot (micro fungus isolated); both fields had grown several crops of peas prior to 1923.

A lot of Advanceer seed, tested at another experiment station and reported to the canner as "96 per cent germination, 12 per cent *Ascochyta*," produced plants 10 per cent of which showed slight traces of both light and dark *Ascochyta* spotting and a mere trace of footrot.

Several fields planted from tested lots of Yellow Admiral seed with as high as 18 per cent *Ascochyta* were followed in two localities. All showed more or less light leafspot but no footrot. In one field the plants weakened by *Aphanomyces* rootrot were badly mutilated by deep stem and leaf lesions, many plants being entirely cut off above the ground; in other fields the spotting was of little consequence.

Footrot was noted in several localities where leaf spotting was entirely absent in fields which had grown several crops of peas before. One such field had practically 100 per cent of footrot, although peas had not been grown for two years. Isolations in such cases yielded usually either the micro fungus or *Fusarium martii* App. and Wr. var. *pisi* F. R. Jones.

These field observations indicate that species of *Ascochyta* were of no great importance as pea parasites in Wisconsin during 1925 and 1926, and at the same time throw some light upon factors which determine the field occurrence of these fungi. Light spotting, produced by *Ascochyta pisi*, was very clearly and closely correlated with the planting of infested seed. Dark spotting was correlated with the occurrence of *Mycosphaerella pinodes* on

the seed planted, and also with the former growth of peas in the field. This fungus shows more marked tendency to accumulate in the field with repeated cropping; probably the perithecial stage functions importantly in overwintering. It is interesting to note that the serious outbreaks of *Ascochyta* on peas recorded in the literature appear to have been concerned with this rather than with the authentic *A. pisi*. Footrot was found to be correlated with the occurrence of either the micro fungus or *M. pinodes* on the seed, but also, and perhaps more importantly, with the previous growth of peas in the field, even beyond a two-year interval between crops. In this behavior the micro fungus agrees with the other pea rootrot parasites. In culture this fungus behaves as a somewhat better saprophyte than either of the species, and its stronger tendency to produce large chlamydospores with a reduced pycnospore production suggests a better adaptation to subterranean existence.

Disease does not necessarily result from the planting of infested seed. The plot trials reported in this paper, as well as the practical field experience of Wisconsin pea growers, demonstrate that seed carrying any of these three fungi may be planted, under some conditions, without the development of an appreciable amount of either leafspot or footrot. On the other hand, it is certainly preferable to plant seed which is known to be free from *Ascochyta* when such seed is available. Seed bearing *Ascochyta pisi* is less to be feared than seed which carries either *Mycosphaerella pinodes* or the micro fungus.

It is clear, from our own observations and from records in the literature, that weather conditions prevailing during germination and the growth of the crop determine very largely the extent and severity of development of disease from infested seed. A detailed study of the influence of environmental factors upon various phases of the parasitism of these fungi should go far in explaining the irregular behavior of the species of *Ascochyta* parasitic on the pea.

SUMMARY

1. Two species and a provisionally designated form of *Ascochyta* are reported as parasitic on the pea (*Pisum sativum* L.). Two of them have been regarded formerly as *Ascochyta pisi* Lib., the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone.
2. The fungus here regarded as *A. pisi* differs from the imperfect stage of *M. pinodes*: (a) in size and shape of pycnospores; (b) in character of the spot produced on the host; (c) in length of incubation period on aerial parts; (d) in the ability of *M. pinodes* but not of *A. pisi* to cause footrot; (e) in rate and character of growth and sporulation in pure culture on artificial media; and (f) in the failure of ascospores of *M. pinodes* to produce *A. pisi* in culture.

3. A third fungus, related to the imperfect stage of *M. pinodes* and here referred to as the "micro" fungus, is characterized by small and commonly nonseptate pycnosporos. It is a frequent cause of footrot, and may also produce leafspots which are indistinguishable from those caused by *M. pinodes*.

4. Emended descriptions are given of *A. pisi* and the pycnidial stage of *M. pinodes*. The "micro" fungus is described under the tentative name, *Mycosphaerella pinodes* (Berk. and Blox.) Stone, micro form?

5. *M. pinodes* rather than *A. pisi* seems to have been the cause of the more severe outbreaks of the Ascochyta disease on peas reported in literature. Inoculation studies and field observations recorded here indicate that this is a more serious pathogen than *A. pisi*.

6. Seed tests have shown that all three of these fungi are carried on pea seed. *A. pisi* was most frequently present in the samples tested during 1926.

7. The planting of infested seed may serve to establish any of these fungi in the resulting crop of peas, but the extent and severity of the resultant injury may vary widely with environmental conditions.

8. In one season's plot trials, seed infested with these fungi gave, on the average, poorer stands than clean seed, but resulted in no considerable footrot or leafspot.

9. Field observations indicate that *M. pinodes* survives Wisconsin winters better than *A. pisi*, and is more likely to increase with intensive pea culture. The "micro" fungus may persist in the soil for more than two years between crops of peas. The occurrence of *A. pisi* is more closely associated with the use of infested seed.

10. Extended field observations indicate that the diseases caused by species of *Ascochyta* were of minor importance in pea culture in Wisconsin during 1924, 1925 and 1926.

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EXPLANATION OF PLATES

PLATE XVI

Photographs of naturally occurring leafspots caused by "light" (*Ascochyta pisi*) and "dark" (*Mycosphaerella pinodes*) species of *Ascochyta* on leaflets of cultivated pea. All photographed $\times 8$. A, B, and C. "Light" leafspots. D, E, F, and G. Types of "dark" leafspots.

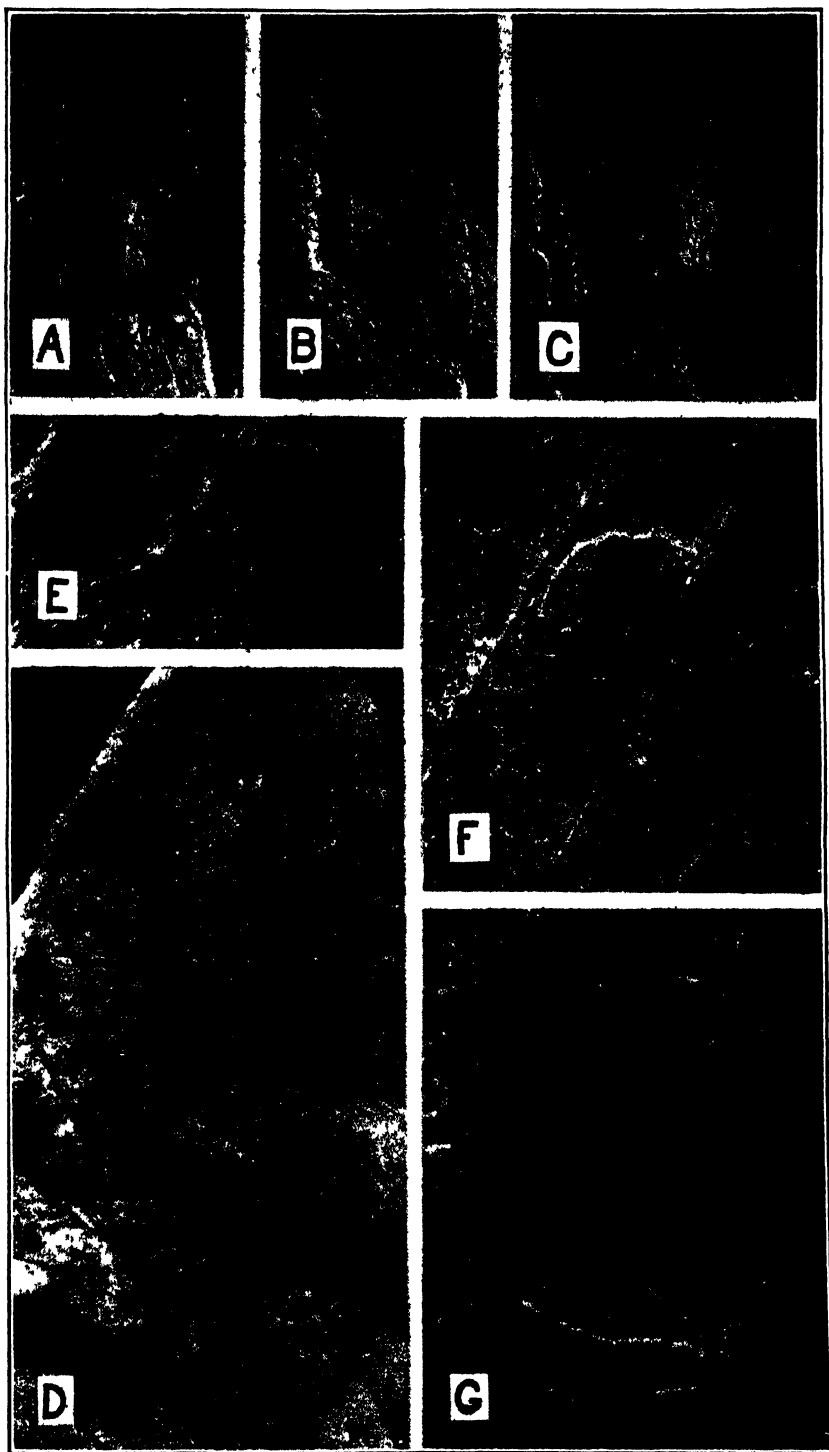
PLATE XVII

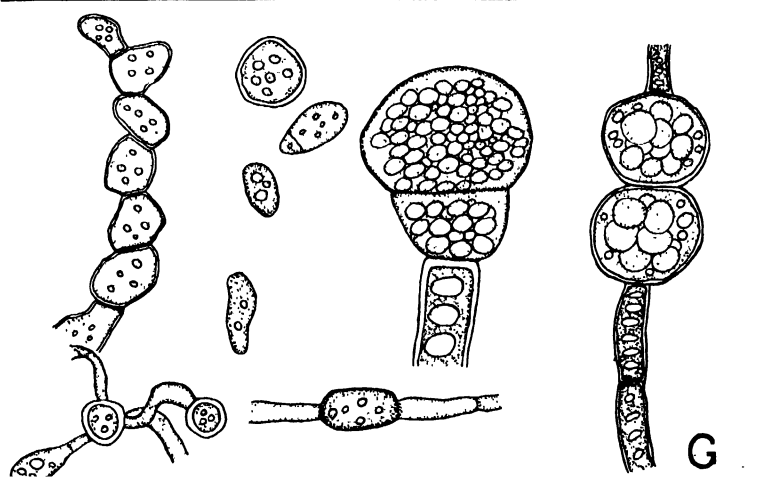
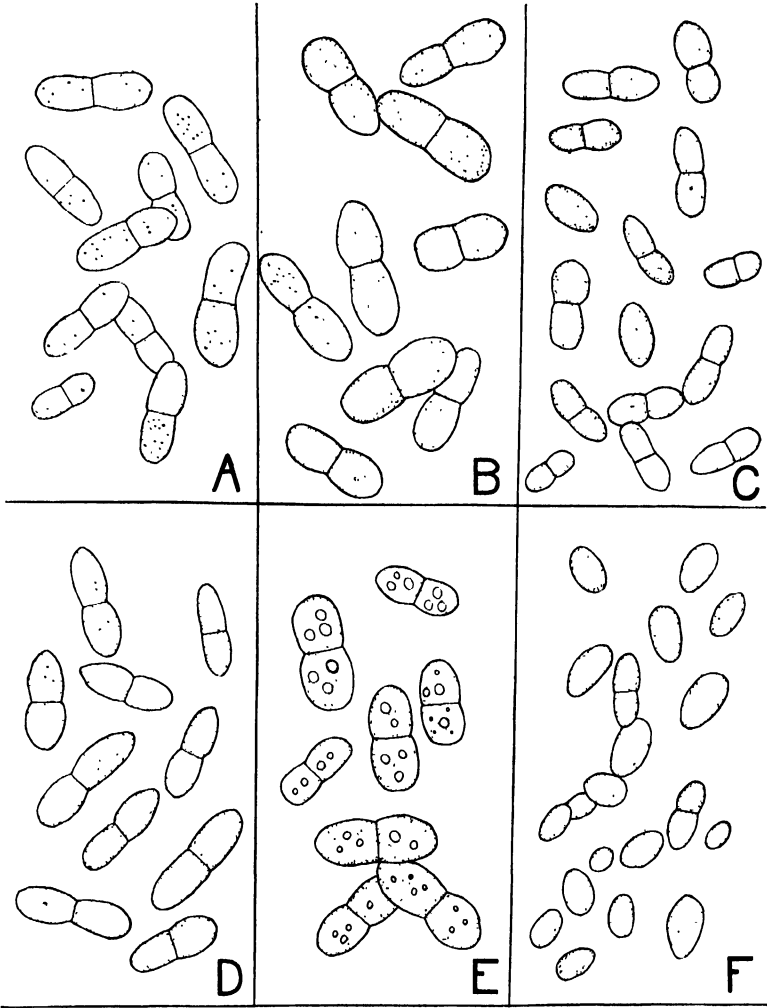
Camera lucida drawings of spores of the pea-infesting species of *Ascochyta*, $\times 1100$.

A, B, and C. Pycnosporos of "light" (*A. pisi*), "dark" (*M. pinodes*), and "micro" fungi respectively, from naturally occurring spots on pea leaflets.

D, E, and F. Pycnosporos of "light," "dark," and "micro" fungi, respectively, from cultures on potato dextrose agar.

G. Types of chlamydospores from culture of "micro" fungus on potato dextrose agar.





LEAFHOPPER INJURY TO CLOVER¹

E. A. HOLLOWELL, JOHN MONTEITH, JR., AND W. P. FLINT

Throughout most of the clover belt a yellowing, reddening, browning, and generally unthrifty appearance of red clover plants (*Trifolium pratense*) is commonly observed. This condition usually becomes evident soon after the first cutting of clover is harvested, becomes more noticeable as the summer advances, and disappears during the early fall. Several attempts have been made to isolate a causal organism from the browned portions, but no fungous or bacterial parasite has been found to be consistently associated with these particular symptoms. The browning of the leaves has been frequently ascribed to "sun scald," while the yellowing and dwarfing have usually been regarded as a physiological response to unfavorable climatic conditions of summer.

The tip and marginal browning of the leaves has often suggested the similar injury found on potato plants which has been attributed to leafhoppers.² The accompanying yellowing and dwarfing suggests the symptoms of "yellows" or "yellow top" of alfalfa which in certain parts of the country is popularly attributed to leafhoppers. Piper,³ in 1914, noted that "a species of leafhopper seems to be constantly associated with alfalfa yellows." However, this correlation was not confirmed by experimental methods until the recent tests by Jones and Granovsky⁴ (work conducted independently at Wisconsin simultaneously with the work here reported on clover). Several species of leafhoppers are frequently found in large numbers feeding upon clover, but no experimental work has been reported heretofore which correlates these symptoms on clover with leafhopper injury.

SYMPTOMS

The leaf injury on red clover, as commonly found, consists of a distinct tip or marginal browning, where the tissue is killed. The "tipburn" is ordinarily V-shaped, with the apex of the V on the midrib, and bounded by the veins extending from this point to the margin. Such dead segments

¹ Investigations conducted cooperatively between the Offices of Forage Crops, Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Illinois Natural History Survey.

² Ball, E. D. The potato leafhopper and the hopperburn it causes. Wis. Dept. Agr. Bul. 23: 76-102. 1919.

³ Piper, C. V. Forage plants and their cultures. 618 pp. New York. 1914.

⁴ Jones, Fred R., and A. A. Granovsky. Yellowing of alfalfa caused by leafhoppers (Abs.). Phytopath. 17: 39. 1927.

are generally distinctly curled. A similar, though smaller, browned area may occur anywhere along the leaf margin. Frequently the entire leaf may wilt, dry out and become brown. At times, instead of this browning, the affected leaves may become yellowed or bronzed, as in alfalfa "yellow-top," this symptom being particularly common in some of the European strains of red clover. There is often a distinct distortion of the stems and petioles. When growing parts are affected, development is decidedly checked and in severe cases the plants are killed.

In the early summer of 1926 plants of red clover which had just reached the blossoming stage in pot cultures at the University of Illinois were found to be generally affected with this marginal and tip burning of the leaves, together with the yellowing and dwarfing. It was observed that these plants were heavily infested with leafhoppers, mainly the potato leafhopper (*Empoasca fabae*, Harris). The abundance of these insects and the close similarity between symptoms manifested on these clover plants and those described as hopperburn on potatoes further served to support the supposition that the tip and marginal browning of these two hosts might be attributed to the same cause. In field observations near Urbana, Illinois, it was noted that, where this type of injury was most noticeable, leafhoppers were usually most plentiful. As a result of these observations, experiments were conducted in insect-proof cages to determine whether this condition of clover was actually due to leafhopper injury.

FIRST TEST—URBANA, ILLINOIS

Four red clover plants of the Tennessee anthracnose-resistant strain, F. C. I.⁵ No. 2469, and two plants from English seed, S. P. I.⁶ No. 61333, were selected as nearly alike as possible in regard to vigor and growth. These plants, which were approximately 8 months old, were sprayed with nicotine sulphate and examined to make sure they were free from insects before placing them in wire insect-proof cages (Fig. 1) on July 20. Two of the Tennessee and one of the English plants were placed in each of two cages. The following day 20 leafhoppers (*Empoasca fabae*) in different stages of development were placed in Cage 2, and on July 27 a few additional hoppers of the same species were inserted to replace some that had died. The cages were examined frequently to observe any injury to the plants and to see that no leafhoppers were present in the insect-free cage.

On August 2, differences were first noted between the plants of the two cages. In Cage 2 several leaves had died, many others were browning and the plants in general lacked vigor and appeared stunted. It was noted that

⁵ Accession number of the Office of Forage Crops, Bureau of Plant Industry, United States Department of Agriculture.

⁶ Accession number of the Office of Foreign Seed and Plant Introduction.

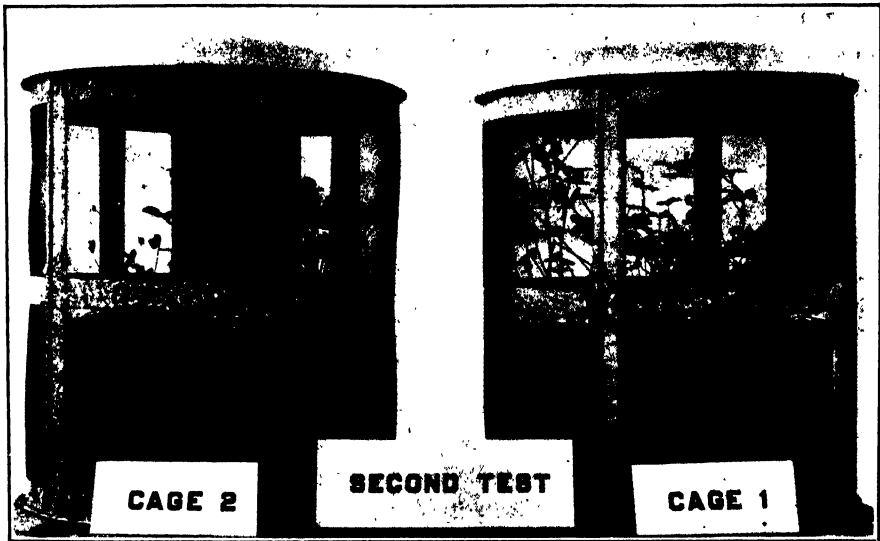


FIG. 1.—Cages used in the experiments at Urbana, Illinois, to test the effect of potato leafhoppers on red clover.

the relatively glabrous English clover was injured to a greater extent than the Tennessee plants. The plants in Cage 1 showed no injury, whereas the extent of damage to those in Cage 2 increased rapidly. On August 19, the plants from both cages were removed and photographed (Fig. 2). Those from Cage 1, which had all remained entirely healthy and had bloomed, were examined for leafhoppers, but none was found. Plants 2 and 3, from the leafhopper-infested cage, were dead. No. 1 had a few green leaves,

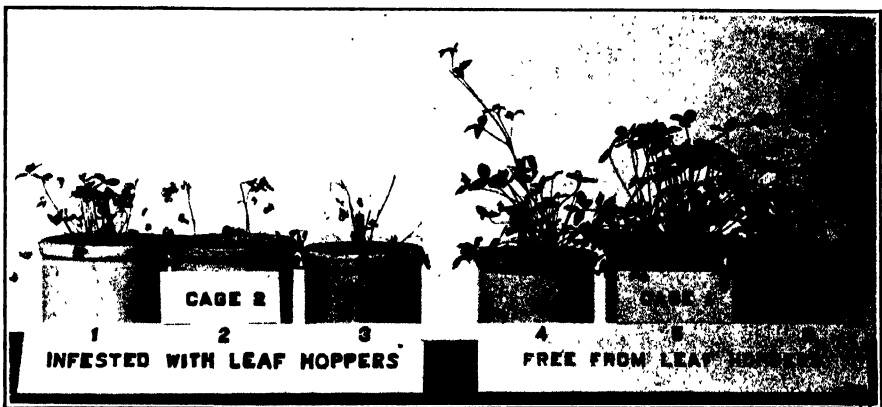


FIG. 2.—First Test. Red clover plants from cages free from, and infested with, leafhoppers. Plants 1, 2, 5, and 6 are from Tennessee anthracnose-resistant seed, F. C. I. No. 2469. Plants 3 and 4 are from English seed, S. P. I. No. 61333.

but did not make further growth, and later died, even though placed under favorable conditions. The dead leaves and stems of each plant were carefully examined for bacteria and fungi, but, except for an apparently saprophytic species of *Alternaria*, no organism was found consistently in the affected tissue.

SECOND TEST—URBANA, ILLINOIS

In order to substantiate the evidence obtained from the first test, the experiment was repeated. On August 19 the three plants from Cage 1, which was free from leafhoppers in the previous test, were placed in Cage 2 to be infested with hoppers in the second test. Three similar plants, from the same seed sources, were placed in Cage 1 to be kept free from insects. About 20 leafhoppers (*Empoasca fabae*) were placed in Cage 2 on August 20, and during the first week of September 10 more were added, for many hoppers had died in the meantime. A mature leafhopper was found in Cage 1 on September 22, and when the plants were removed for photographing on October 2, two mature hoppers and several nymphs were found, showing that the plants therein had not been completely free from hoppers. Plants 2 and 3 from the infested cage were dead, and a few dead stems and leaves were noted on No. 1 (Fig. 3). Plants 4 and 5 showed no injury of

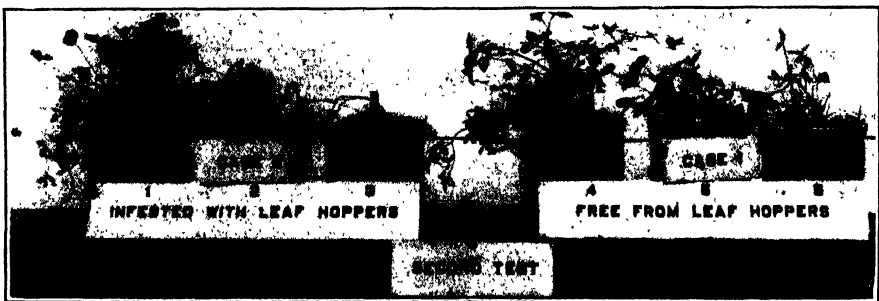


FIG. 3.—Second Test. Red clover plants from cages free from, and infested with, leafhoppers. Plants 1, 2, 5, and 6 are from Tennessee anthracnose-resistant seed, F. C. I. No. 2469. Plants 3 and 4 are from English seed, S. P. I. No. 61333.

any kind, but a few stems and leaves had been killed on plant 6, indicating that the leafhoppers which were found in this cage had been feeding only on one plant or had gained entrance into the cage too late to affect results materially. All the plants in the second test had been cut twice during the season and were less vigorous than those in the first test. Observations made in the second test confirm those in the first, in that the smooth succulent English plants appeared to be injured sooner than those of the hairy American type.

The plants of the potted series, which were not used in these experiments, were sprayed with bordeaux mixture. It was found that, when thus protected, the plants remained comparatively free from leafhoppers, and were much more vigorous and healthy than when heavily infested with the insects.

OBSERVATIONS AT ARLINGTON FARM, VIRGINIA

During the summer of 1926 the same type of injury was observed on the plantings of red clover at Arlington Farm, Virginia, where several species of leafhoppers were abundant. The tip and marginal browning was common on plants grown from seed produced in various foreign and American localities. In general, it appeared that the relatively glabrous foreign clovers were more seriously affected than were the pubescent native strains. Yellowing and dwarfing were much more conspicuous in the plots sown with Italian seed. In these plots the mortality also was greater, but since the Italian strain is more susceptible to certain diseases, and is less vigorous during the summer months than the native strains of red clover, the actual relative losses due to hopper injury have not yet been definitely determined.

In the red clover plots planted with Italian seed there is usually a large number of alfalfa plants. Throughout the summer it was observed that these alfalfa plants were much dwarfed and showed the typical symptoms of "yellows" or "yellow top." Adjacent plantings of alfalfa from seed of different origin were heavily infested with leafhoppers and the plants were stunted and yellow. Several inquiries received by the Department of Agriculture from different eastern localities indicated that alfalfa "yellow top" was more conspicuous than usual during the summer of 1926. This was probably due to the presence of leafhoppers in numbers greater than usual.

The yellowing, bronzing and dwarfing found typically in alfalfa were more pronounced in the Italian red clover than in the native American clovers in which the leaf-browning symptoms predominate. Leafhoppers appeared to be somewhat more numerous on the glabrous Italian red clover and on the alfalfa in these plots than on the plants of the pubescent American strains in adjoining plots. Whether the more striking symptoms manifested in these plants are due to some preference of the leafhoppers for these strains or to greater susceptibility of the plants to this injury was not determined. Plants of Italian and native clover were caged at Arlington, but the differences were not sufficiently striking to warrant any definite conclusions. This failure was probably partially due to the fact that the plants showed some symptoms of injury at the time they were caged and these symptoms persisted in the insect-free cages, and also due to the death

of the majority of the caged leafhoppers. However, there were differences which supported the results of the experiments at Urbana, Illinois.

INJURY ON OTHER CLOVERS

On other species of *Trifolium*, including *T. medium*, *T. hybridum*, *T. repens* and *T. incarnatum*, symptoms similar to those on red clover are commonly in evidence during the summer months and could be associated with large numbers of various species of leafhoppers. The symptoms vary somewhat with the species; in *T. medium*, for example, the tip burn is much more conspicuous, whereas in *T. hybridum* the yellowing or bronzing of the leaves is most noticeable. Other legumes used as forage crops frequently exhibit similar yellowing or tip burning and, since leafhoppers are usually numerous on such crops, it may be assumed that this condition is caused in large measure by these insects.

UNIVERSITY OF ILLINOIS,

AND

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U. S. DEPARTMENT OF AGRICULTURE.

THE FLAGELLA OF *BACILLUS AMYLOVORUS*

MARY K. BRYAN

In a recent publication¹ Dr. H. R. Rosen questions the peritrichiate flagellation of the fire-blight organism, *Bacillus amylovorus*, and figures polar flagella as demonstrated by a stain of his own devising. He claims that the bacteria with which he worked were isolated by him from typical fire-blight lesions, agreed with *B. amylovorus* in cultures, and produced infections on young pear fruits.

Again in a later paper² Rosen says "Likewise it has been determined that the germ causing the disease known as fire-blight of apples and pears has usually but a single polar flagellum and in no case has it been found to have appendages arranged around the body of the organism."

In the summer of 1926 the writer obtained *Bacillus amylovorus* from blighted twigs sent by pathologists from as widely separated places as New York, Illinois, Georgia, Maryland and the District of Columbia. Three of these were on pear, two on apple and one on *Crataegus*.

The flagella of organisms from these six isolations have now been stained by the method of Casares-Gil. A colony which proved very virulent when inoculated into pear twigs was used in each case.

The results are shown in plate XVIII. As with other capsulate organisms it has been difficult to get good clean slides. Peritrichiate rods were always numerous on every slide stained from all sources. Some rods, however, show only one flagellum. These are easy to find on preparations of any multiflagellate pathogen just as many detached flagella and many rods with no flagella are commonly present.

It seems probable, therefore, that Rosen either did not have the right organism or that his bacteria lost most of their flagella in the process of staining.

LABORATORY OF PLANT PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

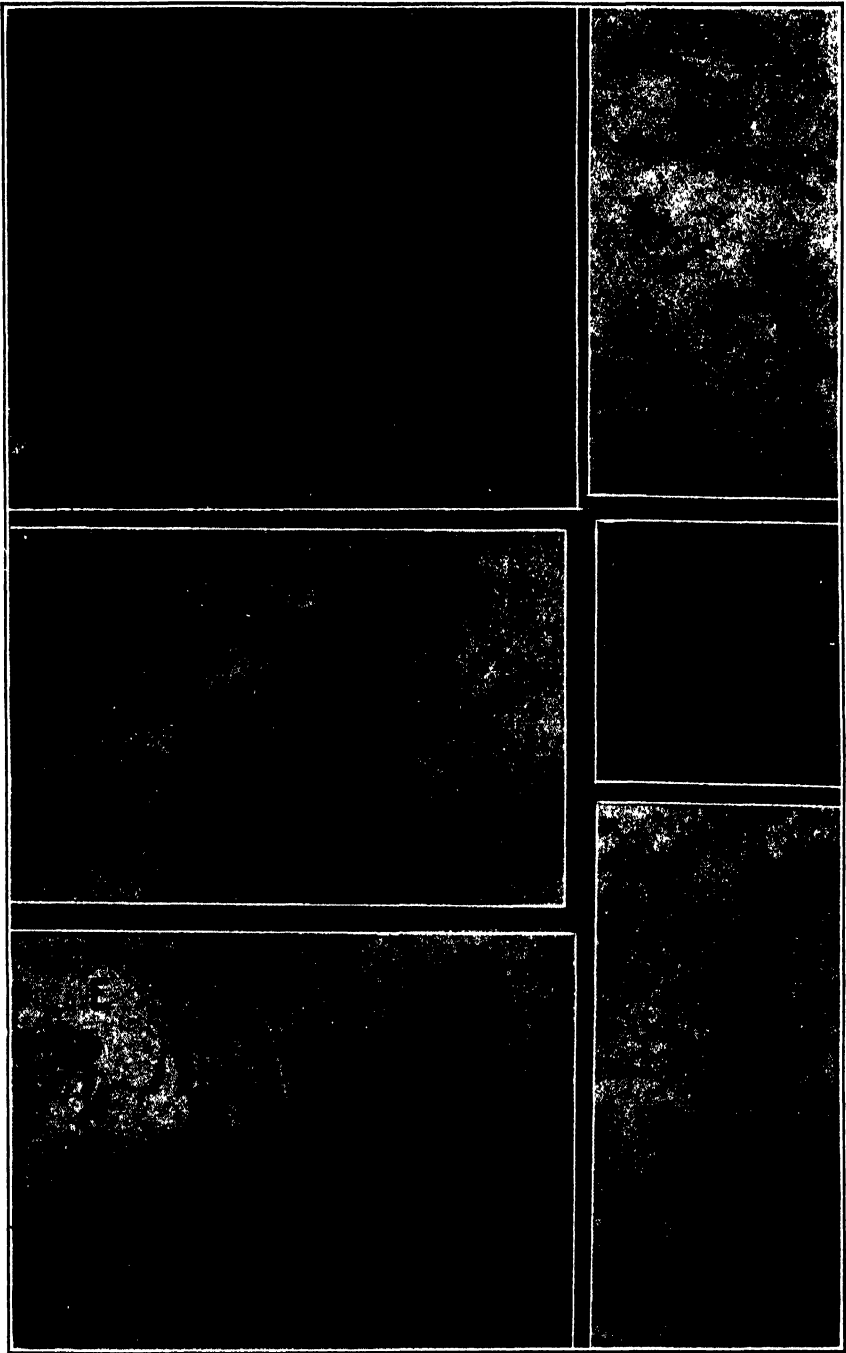
¹ Rosen, H. R. The number and arrangement of flagella of the fire blight pathogen, *Bacillus amylovorus*. *Mycologia* 18: 23-26. 1926.

² Rosen, H. R. Plant Diseases: Morphology and life cycles of bacteria. 38th Ann. Report, Arkansas Agr. Exp. Sta. Bul. 215: 58. 1926.

EXPLANATION OF PLATE XVIII

Bacillus amylovorus from six sources, stained by Casares-Gil's flagella stain. Note the capsules. Photomicrographs by James F. Brewer.

- A. Pear twig from New York.
- B. Apple twig from New York.
- C. *Crataegus* from the District of Columbia.
- D. Pear twig from Georgia.
- E. Pear twig from Illinois.
- F. Apple twig from Maryland.



THE CURLY TOP OF SUGAR BEET IN THE ARGENTINE

G. L. FAWCETT

When the curly top of sugar beet was first noticed in the Argentine a few years ago, it was taken for granted that it was the same as the North American curly top, and it was believed that *Eutettix tenella*, which transmits the disease in that country, would occur here. However, no insects agreeing closely with the description by Ball¹ were to be found. The two species of leaf-hoppers differing least from *E. tenella* were sent to the taxonomist for this group at the California Academy of Science and to the United States National Museum. In a reply received from the latter institution, it was stated that one of the insects was a dark form of *Aceratogallia sanguinolenta* Prov., and the other, *Eutettix* sp. Meanwhile the so-called *A. sanguinolenta*, gathered on diseased beets, had been shown to transmit the infection of curly top to healthy beets grown under cages. The beets under cages into which the *Eutettix* sp. and other insects were introduced remained healthy, as did the checks. A description of this work was published in 1925.² Recently a letter was received from Dr. Herbert Osborn, to whom specimens were sent not long ago, in which it was stated that my *A. sanguinolenta* is really *Agallia sticticollis* Stål., a distinctively South American insect; it would now be placed in the *Aceratogallia* of the later classification.

No doubt it is because of the interest taken in curly top in North America, where it causes much loss to the sugar industry, that my paper received unexpected attention. The fact that the transmission of the disease was attributed to *A. sanguinolenta*, an insect proved to have nothing to do with the transmission of curly top in the United States, must have seemed rather startling. A letter was received from Dr. Eubanks Carsner, in which he suggested the desirability of a more authoritative determination of the insect, which we proceeded to obtain. He also expressed his belief that *E. tenella* was the real cause of our curly top, kindly supplying photographs and descriptions which would have made it difficult not to recognize that insect had it occurred here. Of course the accidental introduction of *E. tenella* into the cages along with other insects would have furnished sufficient explanation of the occurrence of the disease in the cages. But this

¹ BALL, E. D. The leaf-hopper of the sugar beet. U. S. Dept. Agr., Bur. Entom. Bul. 66. 1909.

² FAWCETT, G. L. Enerespamiento de las hojas de la remolacha azucarera. Revista Industrial y Agrícola de Tucumán 16: 39-46. 1925.

criticism does not take into account the relative ease with which the different species of leaf-hoppers can be distinguished from each other, even with the naked eye. My practice has been to collect the insects a few at a time, in a test-tube. In the case of the earlier experiments the insects were then examined with a hand lens before putting them into the cages. This year the insects have been etherized in the test-tube, and then removed and examined separately with a microscope of a magnification of about 30 diameters. I do not know that etherizing has been used before in such work. It facilitates examination of the insects and, if prolonged exposure is avoided, does not injure them.

The experiments carried out this season have confirmed the results obtained previously. In addition to the larger cages of cloth and wire, under which the beets were planted and grown, we have also made use of smaller cages, each covering a single plant in a pot. In all cages into which the *A. sticticollis* has been introduced, curly top has developed; the plants in the check cages and in those into which other leaf-hoppers were introduced have remained healthy. *Agallia sticticollis* Stål is beyond all doubt the agent of transmission of sugar beet curly top in the Argentine. A complete account of this later work will be published in *Revista Industrial y Agrícola de Tucumán*.

Had *Eutettix tenella* been found here even in small quantities, it would have been tried out in the experiments the same as the other leaf-hoppers. Mr. Chas. F. Henderson, of the U. S. Department of Agriculture, who is studying the parasites of *Eutettix tenella* in South America, made a careful search for the insect in Tucumán this summer but did not find it. He tells me that it is not likely to be found in regions of such heavy rainfall as occurs in this province.

ESTACIÓN EXPERIMENTAL AGRÍCOLA DE TUCUMÁN,
TUCUMÁN, ARGENTINA

ON THE USE OF THE TERMS SAPROPHYTE AND PARASITE

F. L. STEVENS AND P. A. YOUNG

The terms parasite and saprophyte have long been used in plant pathology. As most authoritatively defined, they signify as follows:

Parasite. Webster's New International Dictionary, 1923: "Biol. A plant or animal living in, on, or with, some other living organism (called its host) at whose expense it obtains its food, shelter, or some other advantage." Webster's Unabridged Dictionary, 1894: "(a) (Bot.) A plant obtaining nourishment immediately from other plants to which it attaches itself, and whose juices it absorbs; sometimes, but erroneously, called epiphyte. (b) A plant living on or within an animal, and supported at its expense, as many species of fungi of the genus *Torrubia*." Funk and Wagnalls, 1917: "A living organism, either an animal or a plant, that lives on or in some other living organism, known as its host, from which it derives its nourishment for the whole or a part of its existence, as a louse, tapeworm, mistletoe, or dodder." Century Dictionary: "Those which feed on living organisms are termed parasites."

Saprophyte. Century: "Bot. A plant that grows on decaying vegetable matter." Funk and Wagnalls, 1917: "An organism that lives upon dead organic matter, as certain fungous or other plants without chlorophyll, various bacteria, etc." Webster, 1923: "Biol. Any organism living on dead or decaying organic matter. . . ."

These current, popular definitions of the dictionaries do not coincide with technical usage. Thus, the entomologists do not regard insects feeding on or in plants as parasites, nor are mosquitoes or horse flies parasites, in their usage, because they are not constantly attached to their hosts.

The qualifying terms obligate and facultative were introduced with obvious significance: obligate parasites deriving food only from living organisms: facultative, sometimes from living organisms.

There is daily need in plant pathology of terms to designate several categories of parasitism which do not coincide precisely with any of those mentioned above, and for which there are no terms in existence or at least in general use. A few examples representative of large classes of parasites are as follows:

(A) An organism like *Venturia inaequalis* is strictly saprophytic during its entire ascigerous period, which is about half of the year. During the other half of the year it is naturally parasitic, yet it germinates *in vitro* and grows well in non-living culture media. Is *Venturia inaequalis* therefore a saprophyte? Is *Fusicladium dendriticum* an obligate parasite?

B. The converse of group "A" (hypothetical): the ascigerous stage is parasitic and the conidial stage is saprophytic.

C. *Ustilago zaeae* and *U. avenae* are usually called obligate parasites; yet their sporidia sometimes grow indefinitely as saprophytes and therefore cannot be ignored. Are they then obligate parasites, facultative saprophytes, or just saprophytes?

D. A vast number of pathogenic fungi do not at all, or for only a brief period in their tenancy of the host plant, live in the living tissues of the hosts; on the contrary, they send ahead a sapper van of toxins or enzymes that kills the host cells before the arrival of the fungus. Examples are *Phytophthora infestans*, *Botrytis*, and numerous other fungi. Are these to be classed as saprophytes?

E. Many fungi possess a mycelium that wanders over the surfaces of the leaves or in the middle lamellae, and sends haustoria into the living cells. Even the haustoria do not attack living protoplasm in many cases, although they may penetrate it to reach the non-living cell contents. Are the powdery and downy mildews really parasites?

F. *Fusarium* gains access to plant tracheae, for example, through a hydathode or a root wound. It multiplies in the non-living contents of the tracheae and causes the death of the host, although it may spend no part of its life in the living tissues of the host. *Pseudomonas campestris* also exemplifies this phenomenon. Are these organisms parasites at all? Consideration of the preceding cases leads to the following conclusions:

Organisms whose presence causes diseases in plants, even though they do not directly attack living plant parts, may properly be called pathogens. (The use of the final "e" in pathogene is no more necessary here than it is in the correlative terms oxygen, hydrogen, etc. Murray's Dictionary gives the spelling as "pathogen," although the Standard Dictionary gives it only as "pathogene.")

But can all these organisms under current, standard, accepted definitions be termed parasites? Indeed, it would appear that few organisms other than the Chytridiales are strict parasites. Shall the definition of parasite be modified or new terms be introduced? We favor the former solution and suggest the following definitions: A parasite is an organism which lives in or is attached to some other species of living organism from the living matter of which it secures part or all of its food materials. A saprophyte is an organism which secures its food from dead organic matter.

Tentatively accepting these definitions, we may denominate as natural parasites such organisms as are parasites in nature, but which can be grown artificially on agar, in culture media in tubes, etc.; or these organisms may be directly designated as artificial saprophytes, or, indeed, as natural parasites (artificial saprophytes). Obligate parasite should mean, as it does now

in definition though not in usage, that an organism is a natural obligate parasite with no artificial saprophytism. A correlative group is artificial parasites (natural saprophytes).

Organisms that are parasitic in both perfect and imperfect stages may be called *totoparasites*. The name *tropoparasite* may be applied to those organisms in which the conidial stage is parasitic and the ascigerous stage is saprophytic. If desirable for clearness, the term *teleutoparasitic* might be used to designate organisms that are parasitic in the perfect stage, and *deuteroparasitic* might be applied to those that are parasitic in the conidial stage.

One other group must be considered: Organisms sometimes parasitic that may in a given stage live as saprophytes, in the soil, for example, or within living host plants. Examples of such are *Fusarium* and various bacteria. Obviously these organisms are readily classified under the old term facultative parasite.

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PHYTOPATHOLOGICAL NOTES

Beef Infusion Versus Beef Extract Media. The conviction has recently been forced upon the writer that not all plant pathologists realize the differences between beef extract media and beef infusion media. Beef extract is now standard in many laboratories, but much early work was done with beef infusion and some pathologists still prefer it for routine work.

Since until recent years Fuller's scale was used to record acidity, this must be used in comparing present work with that of earlier workers. Quirk and Fawcett¹ have shown very clearly in their paper—which, because of its place of publication, probably has not reached the attention of all pathologists—that Fuller's scale values in the two media represent vastly different pH values, and that pH values determine growth. While in the highly buffered beef infusion media +32 Fuller's scale with a pH value of 5.2 represents the limit of acid toleration for many pathogenes, the same pH in the unbuffered beef extract media is only +13 Fuller's scale. A worker comparing the toleration of acid by *Bact. pruni* in beef extract media did not understand why it would not grow at +28 as recorded by an earlier worker, who doubtless used infusion media. The present writer grew the organism in question in extract and infusion media for comparison; the limit of growth was found to be +29 (pH 5.3) in infusion media but +13 (pH 5.3) in extract media.

Another difference between the two media is their influence on the formation of green fluorescence. Here again a pathologist finding no green coloring in his beef extract cultures of *Bact. citriputeale* explained the fact that an earlier worker always found green fluorescence with the same organism by supposing that it was a difference in strains of the organism. With this organism the writer tested the two media and found that the greening occurred on beef infusion, not on beef extract.

Other organisms recorded as green fluorescent were then tested on agar slants of the two media with various pH values. The organisms used were *Bact. marginatum*, *Bact. tabacum*, *Bact. syringae*, *Bact. vignae* and *Bact. delphinii*. The following results were obtained.

On beef extract peptone agar a slight greening which ordinarily would go unnoticed was observed with all the organisms in pH 8.0 to pH 8.5 which is beyond the range usually used in routine work, but no greening either above or below this. On the other hand, on beef infusion peptone agar with all the organisms used, green fluorescence was very evident promptly and continuously in pH 6.5 to pH 9.0, strongest in pH 7.0 to pH 8.0.

¹ Quirk, Agnes J., and Edna H. Fawcett. Hydrogen-ion concentration vs. titratable acidity in culture mediums. *Jour. Infectious Diseases* 33: 1-59. 1923.

With one organism, a strain of *Bact. syringae*, a decided surface wrinkling occurred constantly on beef infusion agar, while on beef extract agar the surface of colonies was always smooth.

It is important, therefore, for all workers to state which medium they have used. Moreover, even though beef extract is used as the standard medium, beef infusion agar is a better differential medium for green fluorescence and for some other cultural characters.—MARY K. BRYAN, Laboratory of Plant Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture.

An Emendation of the Description of Ophiobolus heterostrophus.—On my account¹ of *Ophiobolus heterostrophus*, a fungus causing a leaf spot of maize in many of the warmer regions of the world, the filamentous ascospores were described in part as being typically four in number and disposed in multiple heterostrophic helicoid arrangement. In connection with experimental work subsequently undertaken to determine the conditions involved in the development of the ascigerous stage, some lots of material were obtained in which each ascus regularly contained eight spores, the larger number of spores entailing a corresponding increase in the volume of the containing structures. In somewhat exceptional instances, also, a reversal in direction of rotation of the spores was observed in the basal portion of the ascus, although such cases were hardly frequent enough to render the specific name of the parasite generally inappropriate. While reversal in direction of rotation may probably best be interpreted as a casual irregularity, the inconstancy in number of ascospores would seem more likely to be related to variation in basic developmental processes. Whatever explanation may be attached to these features as a result of further study, they are pointed out in the present note mainly for the purpose of emending the morphological definition of the species. The emendation is of interest especially because the ascigerous stage under consideration is the only one hitherto recorded as associated with any species of that type of *Helminthosporium* which is distinguished by the bipolar germination of ellipsoidal conidia.—CHARLES DRECHSLER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Powdery mildew of muskmelon.—Dr. I. C. Jagger, in *Phytopath.* 16: 1009–1010. 1926, reports on “Powdery mildew of muskmelon in the Imperial Valley of California in 1925” and states that perithecia of the fungus have not been found. The writer of the present note just happened to read S. U. Shambel’s note on “*Sphaerotheca fuliginea* (Schecht.) Pall. on Muskmelon” in the Russian journal “Diseases of Plants,” 51–52, 1926. Shambel

¹ Drechsler, C. Leafspot of maize caused by *Ophiobolus heterostrophus*, n. sp., the ascigerous stage of a *Helminthosporium* exhibiting bipolar germination. *Jour. Agr. Res.* 31: 701–726. 1925.

reports that some immature perithecia of muskmelon powdery mildew were found in 1923 in southeast Russia and numerous mature perithecia in 1925, and that the fungus was determined to be *Sphaerotheca fuliginea*.—C. D. SHERBAKOFF, University of Tennessee Experiment Station, Knoxville, Tennessee.

The summer meeting of the American Phytopathological Society.—The 1927 annual summer field meeting and tour will be held in northern Ohio, August 16–19. It is planned to assemble at the Ohio Agricultural Experiment Station and spend Tuesday forenoon, August 16, visiting the experimental disease plots on vegetables and fruits. The afternoon of the first day will be spent in the extensive muck regions around Lodi and Willard. These mucks have been under continuous cultivation for over 25 years, and diseases of all the crops grown there are abundant and severe.

The second day of the tour will be spent in the vegetable and small fruit sections around Clyde, and Catawba Island. Experimental disease plots of tomatoes, potatoes, pickles, sweet corn, peas, sugar beets, raspberries, grapes, and peaches will be visited. In the evening of the second day a boat trip has been arranged for a visit to (a) Kelly's Island, where the experimental plots for the control of mildew and black rot will be studied, and to (b) South Bass Island, where the party will have dinner and lodging.

The third day the tour will continue along the lake to Cleveland. The numerous stops will include a study of raspberry, cherry, and vegetable diseases.

The fourth day the group will be split up according to interests. One party will visit the extensive raspberry propagating region southeast of Cleveland. This part of the tour will be under the supervision of Mr. Wilcox, who will show the disease-free raspberry plantings. The second group will visit the Painesville nurseries and Dr. Bingham's orchard, the largest apple orchard east of the Rockies.

The third group will accompany Mr. Tilford to the large potato section southeast of Cleveland.

The tour has been arranged to show as many of the experimental disease plots on fruits and vegetables as possible. It will include a great variety of diseases on these crops. Many points of natural and historical interest will be included, such as Green Springs, the Blue Hole, the islands of Lake Erie, and Perry's monument.

Automobile space will be arranged at Wooster for all not driving. An attempt will be made to send all members of the Society a letter explaining the tour. Should you fail to receive this, however, please feel that you are invited and that the Ohio pathologists will take care of you.—H. V. YOUNG, Chairman of Committee on Local Arrangements, Wooster, Ohio.

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PHYSIOLOGY AND PARASITISM OF *SCLEROTIUM* *ROLFSII* SACC.¹

B. B. HIGGINS

INTRODUCTION

Throughout the warmer sections of the United States, *Sclerotium rolf sii* is one of the most destructive of soil-inhabiting fungi. It attacks a great variety of cultivated and wild plants in the field, killing the plants outright. It is also a common cause of decay in stored roots and vegetables and of cucurbit and other fruits that rest upon the soil. Very often it destroys planted seeds and seedlings and has several times been reported as a serious pest in nurseries.

Plants once attacked by the fungus are nearly always killed; but, unlike the wilt-producing species of *Fusarium*, which usually attack every plant within an infested area, the attack of *S. rolf sii* may be very irregular. In pepper fields the loss may vary from a few scattered plants to as many as 75 per cent. In soil containing large quantities of undecayed organic matter, every plant may be killed; yet susceptible plants have been grown free from attack over long periods in soil containing numerous sclerotia of the fungus. This latter statement may be illustrated by a brief report of observations on a series of 24 pot cultures of pepper plants grown in a compost soil in the greenhouse for one year. Each pot was inoculated with a culture of the fungus on sweet potato plug as soon as the plants were well started. The fungus spread and killed several plants in each pot during the following two weeks. It then formed numerous sclerotia and ceased growth, remaining dormant over a period of six months. Although the sclerotia were viable, they did not germinate. Finally the plants became infested with aphids and, with the accumulation of detritus from the aphids, the fungus entered another period of growth and killed most of the remaining plants. This was followed by a dormant period of three months, and, finally, a third period of growth.

No practical method of control has yet been developed. Crop rotation is of some value in reducing losses; but, on account of the very wide range of

¹ Paper number 23, Journal Series, Georgia Agricultural Experiment Station.

host species, it is difficult to plan a rotation to rid the soil of this fungus. However, the unusual geographical distribution of the fungus, together with some peculiar aspects of its behavior, has suggested that a thorough knowledge of its physiology might lead to an understanding of its wide host range, its limited geographical range, and possibly to a solution of the problem of its control.

The most obvious factors which might affect its geographical range are temperature and soil reaction. The first investigation, therefore, was directed principally toward (1) determination of temperature relations of the fungus, (2) relation of reaction of substratum to growth, (3) reaction changes in the substratum during growth, (4) relation of various organic nutrients to growth, (5) metabolic products of the fungus, (6) relation of the metabolic products to parasitism, and (7) relation of fungous hyphae to the host tissues.

MEDIA

As *S. rolfsii* is a soil-inhabiting fungus, often appearing on plants in soil containing comparatively little organic matter, it seemed probable that it might require considerable quantities of inorganic nutrients for its best growth. However, preliminary trials on Czapek's solution did not result in very satisfactory growth. Following this, a series of cultures was grown on six synthetic media, variations of Czapek's solution and Richard's solution E., as given by Zeller, Schmitz, and Duggar (24). For comparison with these synthetic media, the following were used: bean decoction, decoction from pepper pods, beef-extract-peptone broth, Dunham's solution, 1 per cent peptone solution, and 2 per cent egg albumen solution.

On none of the synthetic media tried did the fungus appear to grow normally. The growth was slow, and few or no sclerotia were formed. When either peptone or egg albumen was added to replace sugar, the growth was much better and sclerotia formed more abundantly, but only slightly better than on the same concentrations of peptone or egg albumen in distilled water. The addition of sodium chloride to the peptone solution seemed to have little effect. The fungus grew rapidly and luxuriantly, and formed abundant sclerotia on the vegetable decoctions and the standard beef-extract-peptone broth. The proportion between mycelium and sclerotia and the general appearance of the growth was quite similar to that seen on various fruits and vegetables in the field. The growth on broth was considerably more abundant than that on a 1 per cent peptone solution. This was doubtless due to the mineral elements in beef extract.

Since some organic nitrogen seemed necessary for normal growth of the fungus, the standard beef-extract-peptone broth² was adopted as the basal

² Beef-extract 3 grams, peptone 5 grams, distilled water 1 liter.

medium for the study of hydrogen-ion relations, and beef-extract-peptone agar³ for the study of temperature relations.

The beef extract, peptone, and sugars used were those especially prepared for bacteriological work by the Digestive Ferments Company. The other chemicals were of the highest commercial purity. Pyrex glassware was used for all liquid cultures. It was cleaned with alkali and acid, thoroughly washed and rinsed twice with distilled water at the beginning of each experiment. The distilled water was steam distilled in a Stokes Automatic Apparatus, lined throughout with block tin.

All hydrogen-ion determinations were made colorimetrically, using standard indicator solutions and a comparator of the Army Medical School model.

TEMPERATURE RELATIONS

For the study of the temperature relations of the fungus, the rate of growth of mycelium on standard beef-extract agar plates was adopted as the criterion. The results thus obtained were checked also against the length of time required for germination of sclerotia.

Standard petri dishes with bottoms as nearly flat as possible were selected and sterilized. Ten cc. of sterile agar was then poured into each plate, which, after hardening, was inoculated with a single sclerotium, placed near the center of the plate. These were held for 48 hours at approximately 25° C. The limits of the colony in each plate were then marked with ink on the bottom of the plate. The plates were distributed to incubation chambers and incubated for 48 hours at the various temperatures. At the end of 48 hours the plates were removed and the average increase in diameter of each colony measured.

Five strains of *Sclerotium rolfssii* (pepper, Irish potato, peach, Cedrus, and snapdragon strains) were used. Two plates of each strain were placed at each of the temperatures. The results for each of the temperatures are

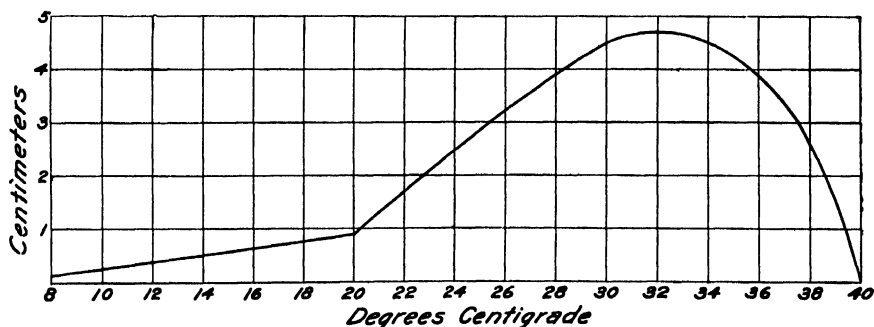


FIG. 1. Increase in diameter of colonies of *Sclerotium rolfssii* on agar incubated for 48 hours at temperatures indicated.

³ Beef-extract 3 grams, peptone 5 grams, agar 15 grams, and distilled water 1 liter.

therefore based on the average measurements of ten colonies. Several lots were incubated at temperatures near the optimum and maximum, and the lower temperatures were checked once. The temperatures used were -10 , -2 , 3 to 5 , 8 , 10 , 15 , 20 , 25 , 27 , 30 , 33 , 35 , 37 , 39 , 40 , 41 and 42° C. The results are shown graphically in figure 1.

For 10 days the fungus did not grow at all at temperatures below freezing or at 3 to 5° C. At 8° C. growth was very slow, only an average increase of 1.46 mm. in diameter in 48 hours; and the average rate over the 10-day period was approximately the same. Fresh sclerotia had not germinated in this chamber at the end of ten days. At 10° C. the sclerotia had germinated at the end of 7 days, and at 15° C. in 4 days. The fungus grew well at all temperatures between 20° and 37° C. Some increase in diameter of the colonies was obtained up to 40° C., but growth soon ceased, and no sclerotia germinated at temperatures above 37° C. The mycelium was not killed by 48 hours exposure to 42° C. When the plates were removed to a lower temperature, vigorous growth soon started again. The minimum, optimum, and maximum temperatures are 8° , 30° to 35° , and 40° C., respectively, with 37° C. the apparent maximum for normal growth of the fungus.

The vegetative mycelium was killed in all cases by exposure to -2° C. for 24 hours, but the mature, ungerminated sclerotia were not killed. Similar results were obtained at the end of 48 hours exposure at -2° C., and at the end of 24 and 48 hours exposures at -10° C. The plates were removed from the freezing chamber and the agar allowed to thaw at room temperature (about 10° C.), then placed in an incubation chamber at 25° C. and left for 48 hours. The mycelium therefore cannot survive freezing, but the ungerminated sclerotia are much more resistant. The effect on sclerotia of alternate freezing and thawing was not investigated.

RELATION OF H-ION CONCENTRATION TO GROWTH

Sclerotium rolfsii is admirably suited to a study of H-ion relations. The sclerotia are large enough to admit of easy handling, fairly uniform in size, and easily freed of hyphae. They float on the surface of liquids, and the slightest growth from the sclerotia can be readily seen. The fungus grows well on beef-extract-peptone media over a wide range of concentrations.

Methods *Us.* —Beef-extract-peptone broth, as recommended by the Committee on the Descriptive Chart of the Society of American Bacteriologists, was used as the standard medium for this study. The broth was made up in 3-liter quantities, titrated, and made neutral to phenolphthalein by addition of N/1 sodium hydroxide solution. It was then divided into 250 cc. lots, and N/1 HCl solution or N/1 NaOH solution added to make each lot acid or alkaline according to Fuller's scale. In this way solutions were made up -5 , -4 , -3 , -2 , -1 , neutral, $+1$, $+2$, $+3$, $+4$, $+5$, $+10$, $+15$, $+20$, $+25$,

+ 30, + 35, + 40, + 45, + 50, + 60, + 65, + 70, + 75 and + 80. Fifty cc. of solution were placed in each of five 100 cc. Erlenmeyer flasks and sterilized. One flask of each set of five was inoculated with a single sclerotium of a strain of *Sclerotium rolfsii* isolated from pepper stems, one with a strain from Irish potato tuber, one with a strain from peach seedlings, and one with a strain from seedlings of *Cedrus deodara*. The fifth was held as a blank for determination of H-ion concentration at the end of the incubation period. All were incubated six weeks at a temperature of 25° C.

The four strains of the fungus were selected from a large number of stock cultures because of the differences noted in the general characters, especially in the size and color of sclerotia. For the inoculations, mature sclerotia were selected from cornmeal agar cultures of uniform age and were handled with a platinum loop to avoid injury. No attempt was made to determine the amount of growth produced in this series because the mycelium extended up the sides of the flasks so that all growth could not be removed. The results are shown in table 1.

TABLE 1.—The effect of six weeks growth of four strains of *Sclerotium rolfsii* on the H-ion concentration of broth adjusted to different initial H-ion concentrations

Initial acidity, Fuller's scale	H-ion concentration after six weeks					Average pH of cultures
	Check. Not inoculated	Culture from pepper	Culture from potato	Culture from peach	Culture from <i>Cedrus</i>	
Neutral	8.30	No growth	No growth	No growth	4.10	4.100
+ 1	8.10	4.10	4.0 +	No growth	Contaminated	4.050
+ 2	7.90	4.10	4.0 +	4.10	4.20	4.100
+ 3	7.50	4.10	4.0 +	4.20	4.20	4.150
+ 4	7.20	4.10	4.0 !	4.00	4.10	4.150
+ 5	6.80	4.00	3.80	4.10	4.00	3.975
+ 10	5.00	4.00	3.80	4.10	4.00	3.975
+ 15	4.10	4.00	3.80	4.10	4.10	4.000
+ 20	3.60	4.20	4.20	4.40	4.00	4.200
+ 25	3.20	4.10	4.00	3.9	4.00	4.050
+ 30	2.80	4.00	3.90	3.9	4.00	4.000
+ 35	2.20	4.20	3.80	3.9	4.00	4.025
+ 40	1.90	4.00	3.20	3.80	4.00	3.600
+ 45	1.70	4.40	3.40	3.80	4.20	3.950
+ 50	1.60	4.20	3.20	3.20	4.70	3.575
+ 55	1.5 +	3.20	2.80	2.80	4.20	3.000
+ 60	1.50	3.00	2.00	2.10	2.60	2.240
+ 65	1.5 -	1.70	1.60	1.50	1.80	1.650
+ 70	1.45	1.50	1.45	1.50	1.50	1.490
+ 75	1.4 -	1.45	1.40	1.45	1.50	1.450
+ 80	1.35	No change				No growth in any strain

There was a slight but constant difference in the relation of some of the strains to H-ion concentration. The Cedrus strain grew in broth neutral to phenolphthalein (pH 8.3); whereas none of the other strains grew beyond pH 8.1. The peach form did not grow beyond +2, pH 7.9. On the acid side, all grew in +75 (pH 1.4-) but not in +80 (pH 1.35). Growth appeared earliest in +10 and +15 (pH 5.0 to 4.1), but the total amounts of mycelium present at the end of six weeks were approximately equal over the range from neutral to about +50 (pH 1.6). In the more acid lots, +55 to +75, growth was slow in starting; the total amounts of mycelium were small; and usually few or no sclerotia were produced, the last character again varying with the strain of the fungus.

On beef-extract-peptone agar the range was somewhat wider. Growth occurred at -2 and +85 Fuller's. The exact H-ion concentrations were not determined.

In broth +1 per cent NaCl the range was somewhat narrowed. The Cedrus strain grew from +3 (pH 7.5) to +70 (pH 1.4), the pepper strain from +4 (pH 7.2) to +75 (pH 1.4-), the potato strain from +4 to +70, and the peach strain in +4 to +60 (pH 1.5). The explanation of this narrowed range doubtless lies in the stronger buffer action of the NaCl broth, which would of course make the medium less readily adjustable to an H-ion concentration favorable for growth of the fungus.

Addition of saccharose to the beef-extract-broth affected quite materially the tolerance of the fungus for both hydrogen- and hydroxyl-ions. The broth was made, neutralized and the normal solution of NaOH or HCl added. It was then flaked, 25 cc. per flask, and sterilized. A 7 per cent solution of saccharose in distilled water was sterilized separately and 10 cc. added to each flask of sterile broth, making 35 cc. of slightly diluted broth containing 2 per cent saccharose.

Growth was obtained in all solutions over the range from -5 (pH 8.8) to +60 (pH 1.4+). The range of tolerance was extended on the acid side and shortened on the alkaline side. Growth started first in solutions of pH 1.6 to 1.5, but subsequent growth was most rapid and greater at lower initial concentrations.

GROWTH OF FUNGUS AND CHANGE IN REACTION PRODUCED ON VARIOUS MEDIA

By reference to table 1, the constancy of the hydrogen-ion concentration may be noted at once. In all broth cultures where good growth of the fungus was obtained, regardless of the initial reaction, the final concentration was near pH 4.0. In the broth with the more acid initial reaction the H-ion concentration was reduced in proportion to the growth of the fungus; in those with an initial reaction between pH 4.0 and the lowest concentra-

tion at which growth was obtained, the H-ion concentration was increased.

Evidently both acid and alkaline substances were among the metabolic products of the fungus on broth; but, in saccharose broth, acids were evi-

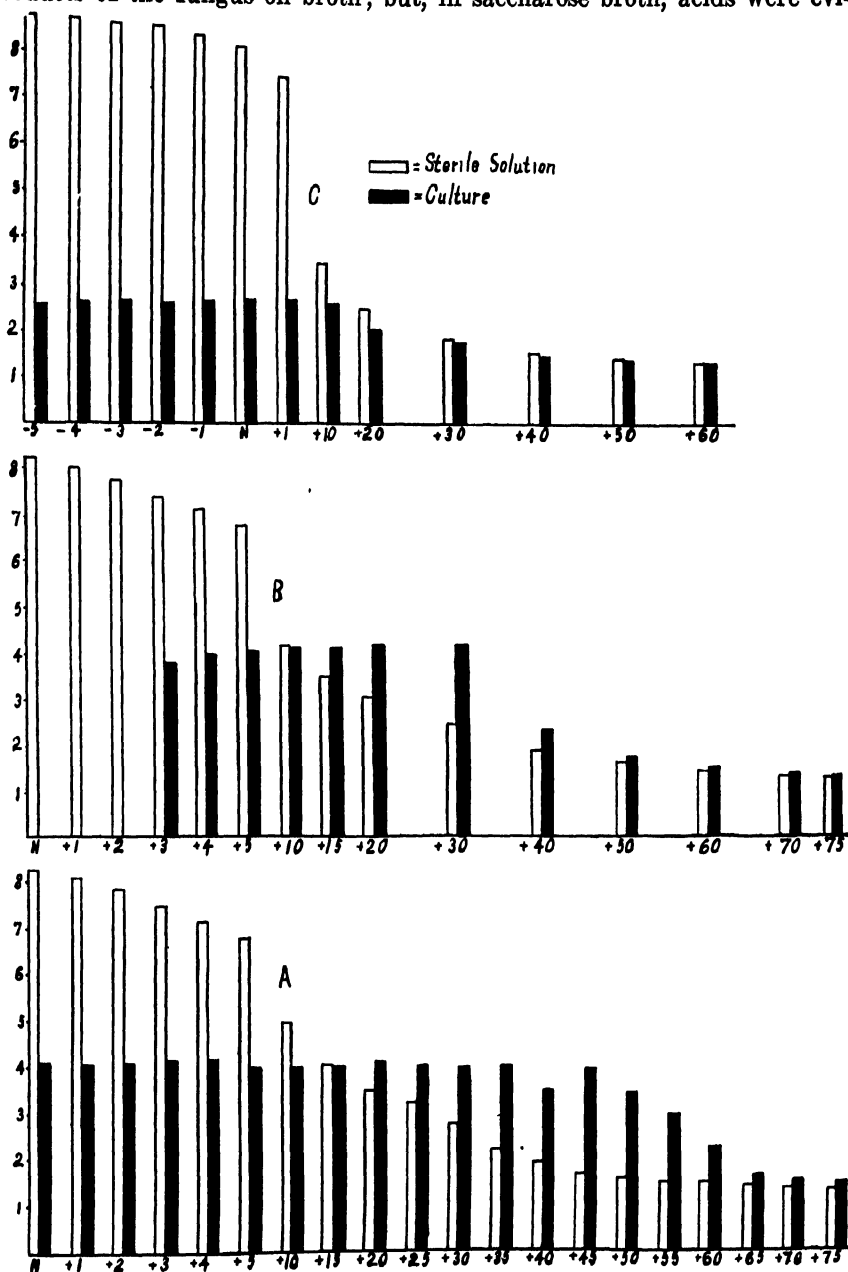


FIG. 2. Changes in reaction produced by *Sclerotium rolfssii* on media of various initial reactions. A, broth; B, broth + 1 per cent NaCl; C, broth + 2 per cent saccharose.

dently produced much faster than bases, as the acidity of all solutions was increased.

This end-point concentration in broth cultures was also independent of the concentration of the nutrients, the amount of medium used, and the depth of the liquid in proportion to the exposed surface. Furthermore, the end point was approximately the same in solutions of any of the proteic substances tried, including broth, peptone, and egg albumen solutions.

When sugar, starch, or other carbohydrate was added to a solution of any of these proteid substances, the end point concentration was very much higher, varying with the amount of carbohydrate added.

The change in reaction of the medium produced by growth of the fungus on beef-extract broth, broth plus 1 per cent sodium chloride, and broth plus 2 per cent saccharose is shown graphically in figure 2, A, B, and C, respectively.

The effect of several other carbohydrates and a few organic acids on the final reaction of broth cultures was tested. The cultures were grown on 50 cc. of medium in 100 cc. Erlenmeyer flasks, and the results are the average obtained from one culture of each of the four strains of the fungus. The results are shown in table 2.

Lactose and glycerin were the poorest of any carbohydrate media tested for growth of the fungus. In broth the lactose appeared to retard growth during the first three to four weeks, and H-ion determinations indicated that lactose was not assimilated during this period of slow growth. However, the pH at the end of six weeks indicated that the lactose was finally assimilated. In milk similar conditions occurred.

These results show further evidence that the assimilation of any carbohydrate by the fungus results in an increase in the acidity of the culture medium. When either oxalic or citric acid is added to a protein medium the acidity is reduced by the fungous growth, a result similar to that noted when inorganic hydrochloric acid is added to a protein medium. There is the difference, however, that the loss in acidity is not so great in the case of the organic acids. The results suggest that the oxalic and citric acids assimilated by the fungus are partly changed into other acids, and excreted again into the culture fluid, although negative tests for the presence of other common organic acids indicate that this is not the case. The most probable explanation lies in the much greater titrable acidity of the organic acid solutions of equal H-ion concentration with hydrochloric acid solutions. Another explanation of the greater change toward neutrality in broth plus hydrochloric acid may be found in the specific action of HCl in changing the proportions of ammonia and of oxalic acid formed from peptone. Results to support this statement are reported under "Metabolic Products."

Citric acid appears to be a very good source of carbon for growth of the fungus. Luxuriant growth was produced on pure lemon juice, and the addition of 2 per cent citric acid to a peptone solution stimulated growth very markedly. A 2 per cent solution of oxalic acid in broth prevented all growth, and even a 1 per cent solution in broth was decidedly toxic. This toxicity appears to be due entirely to free hydrogen-ions in the comparatively highly ionized oxalic acid. That this is the case is shown by comparison with broth plus hydrochloric acid at the same H-ion concentrations.

During the progress of the work it was found that the fungus grew well on a solution of pure saccharose in distilled water. It seemed desirable, therefore, to compare the amount of growth and the final H-ion concentration produced in single nutrient solutions. For this purpose, 2 per cent solutions in distilled water were prepared, flasks 50 cc. per flask, autoclaved, and inoculated with the four strains of *Sclerotium rolfsii* used in the previous experiments. The four cultures and an uninoculated check flask of each solution were incubated 31 days at 25° C. The H-ion concentration of the liquid was then determined, and tests were made for oxalic acid. The mycelium from the four cultures was collected on a weighed filter, washed thoroughly, dried, and weighed, and the weight of the whole divided by four to obtain an average. The results are shown in table 3.

TABLE 2.—The growth of *Sclerotium rolfsii* and the changes in reaction produced on various nutrients

Medium	pH of check	pH of culture	Relative production of mycelium	Relative production of sclerotia
Broth	6.9	4.0	++	++
Broth + 2% saccharose	6.5	2.5	+++	+++
Broth + 3% saccharose	6.2	2.125	++++	+++
Broth + 2% dextrose	6.8	2.75	+++	+++
Broth + 3% dextrose	5.8	2.625	+++	+++
Broth + 3% starch	6.8	2.85	++++	++
Broth + 2% lactose	6.3	2.6	++	+++
Broth + 2% glycerin	6.6	2.5	++	++
Broth + 2% oxalic acid	1.3		No growth	
Broth + 1% oxalic acid	1.4	1.55	+	+
Broth + ½% oxalic acid	2.1	3.175	++	++
1% peptone	7.0	4.175	+	+
1% peptone + 2% mannite	7.0	2.525	++++	+++
1% peptone + 2% citric acid	2.6	3.125	+++	+++
2% egg albumen	7.0	4.1	++	+
2% egg albumen + 2% dextrose	6.4	2.1	++++	++
Separated milk	6.8	2.95	++	+

Slight growth was produced also on 2 per cent solutions of tannic acid, succinic acid, ethyl alcohol, and esculin; but none on acetic, lactic, or oxalic acids, asparagin or brucine.

TABLE 3.—*The growth of Sclerotium rolfsii, the reaction changes produced, and the results of tests for oxalic acid on 2 per cent aqueous solutions of single nutrients*

Media	Weight mycelium mg.	pH, check solution	pH, cultures, average	Occurrence of oxalic acid
Saccharose	47.00	6.5	2.3	present
Dextrose	25.00	4.2	2.4	do
Maltose	66.50	6.4	2.4	do
Lactose	10.00	4.2	2.8	do
Mannite	13.25	7.0	6.3	trace
Amygdalin	40.00	4.0	2.4	present
Citric acid	11.25	2.2	2.2	none
Tartaric acid	7.50	2.0	2.0	do

Oxalic acid was produced in all cultures where the fungus made any appreciable growth, except citric and tartaric acid cultures. In amygdalin cultures, hydrocyanic acid was also produced. The solution had a strong odor of almond oil and a strong Prussian blue color when treated with sodium hydroxide, ferric chloride, and hydrochloric acid. The oxalic acid was probably formed in this case from the glucose of the decomposed amygdalin molecule.

✓ COURSE OF CHANGE IN REACTION OF MEDIUM DURING GROWTH OF FUNGUS

In order to determine the rate of change in reaction of the substratum and the time necessary to reach the end-point reaction, three series were set up in sufficient numbers to allow determination of reaction in one culture daily over a long period of time. For this purpose, standard beef-extract broth was made in 6-liter lots. To one-third of the broth, dextrose was added to make a 2 per cent solution; another third received $\frac{1}{2}$ per cent glucose; and the remainder was used as standard broth. All three lots were then flaked, 25 cc. per flask, autoclaved, and 60 flasks of each lot inoculated with a single sclerotium of the pepper strain. The H-ion concentration of one check flask of each lot was tested (colorimetrically) at this time and one inoculated flask tested each 24 hours during the first 12 days. After this time the change in pH was slower, and tests were made at 48-hour intervals until the sixty-first day. At the close of the experiment, check (uninoculated) flasks were again tested and showed no change in reaction.

The results are shown graphically in figure 3. At the end of the first 24 hours only slight growth from the sclerotia could be seen on broth and on broth plus $\frac{1}{2}$ per cent dextrose, and no change in reaction had occurred. On broth plus 2 per cent dextrose, more growth was evident, and the medium was slightly more acid than at the beginning. After this the reaction changed rapidly toward the acid side—most rapidly in dextrose

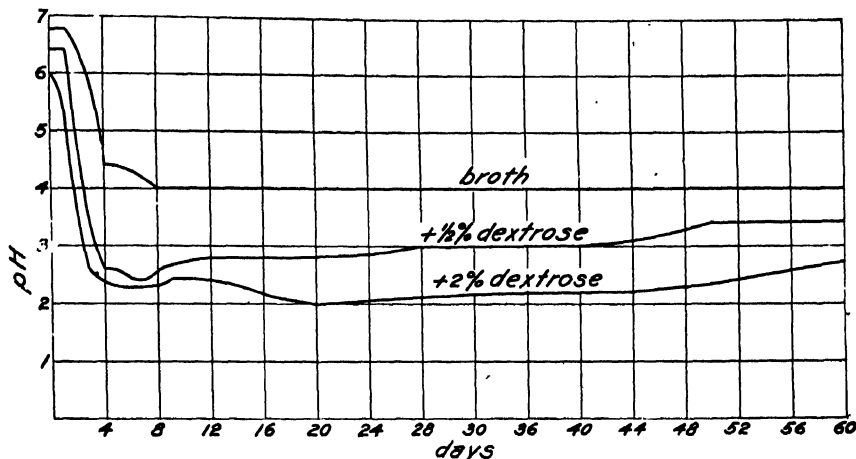


FIG. 3. Curves showing rate and direction of reaction changes produced by *Sclerotium rolfsii* on beef-extract peptone broth, broth + $\frac{1}{2}$ per cent dextrose, and broth + 2 per cent dextrose.

broth—until a maximum acidity was reached. In broth this maximum or end-point, near pH 4.0, was reached on the eighth day and remained constant thereafter. In broth plus $\frac{1}{2}$ per cent dextrose, the maximum acidity, pH 2.4, was reached on the sixth day. Thereafter there was a reversal, and the reaction changed slowly toward neutral once more. In broth plus 2 per cent dextrose, the maximum acidity, pH 2.0, was reached on the twentieth day, after which a reversal toward neutral occurred. In each of the latter, tests for reducing sugars at the time of maximum acidity gave negative results.

A change in the physical state of the medium began also about this time. At the time of maximum acidity most of the liquid of the medium was held in a gelatinous mass about the submerged fungous mycelium. After reversal in the reaction change, this gelatinous mass gradually disappeared, and little remained at the end of the experiment.

Whether or not the reversal in reaction was brought about by assimilation of the acids previously formed, as found by Wolff (23) to occur in cultures of *Bacterium diphtheriae*, has not been determined. At any rate

the reversal was never so complete as in broth brought to an even greater *H-ion concentration* by the addition of hydrochloric acid. The behavior is remarkably similar to that on broth plus $\frac{1}{2}$ per cent oxalic acid in which the change appears to be due partly to natural decomposition of the acid. The fact that the loss of acidity continued after fungous growth had ceased (about the thirtieth day) is further evidence of the latter theory; though this continued change may have been in part due to liberation of ammonia previously held inside the fungous hyphae.

METABOLIC PRODUCTS

The mechanism of the reaction changes produced by fungi has received little experimental study. Most exact cultural studies of the *H-ion* relations of fungi have been made on synthetic media containing inorganic salts plus some organic substance as a source of carbon. Obviously the possible changes due to unequal absorption of the various elements of such solutions and the production of organic acids and ammonia by the fungus were too complicated to allow definite conclusions as to how the change in reaction of the medium was brought about.

Since *S. rolf sii* grew well on standard beef-extract broth, peptone solutions, and to some extent on pure sugar solutions, producing in each a characteristic change in reaction, it seemed possible that an insight might be gained into the nature of metabolism of the fungus by determination of the metabolic products in such simple solutions.

For this purpose a solution of 1 per cent peptone and another of 1 per cent peptone plus 2 per cent dextrose were made up in liter quantities in 2-liter flasks, autoclaved, inoculated with a single sclerotium of *S. rolf sii* from pepper, and incubated at 25° to 27° C. for six weeks. Standard broth solution plus 20 grams saccharose was made up in 500 cc. of distilled water. In another flask 30 grams of pure calcium carbonate were added to 500 cc. distilled water. After sterilization the saccharose broth was added to the calcium carbonate, inoculated and incubated at 25° to 27° C.

A 3 per cent saccharose solution in distilled water was flaked, 100 cc. per flask, and autoclaved. Eight flasks were inoculated and incubated as above. After incubation the fungous growth was removed and the liquid from the eight flasks combined for chemical examination.

All the cultures were tested for various substances by the method of Eyre (5). The results are shown in table 4.

In all media containing peptone the principal products found were ammonia and oxalic acid. Some succinic acid was present in all cultures, but the amount appeared to be small. The same was true of formic acid. The total volatile acid from 400 cc. of the saccharose culture was neutralized by

0.8 cc. N/20 NaOH. The calcium oxalate from the same solution amounted to 140 mg., equivalent to approximately 121 mg. of free oxalic acid. The presence of this trace of formic acid in all cultures without the association of any alcohol or aldehyde would indicate its origin through decomposition of oxalic acid during the two distillations.

Cultures on 1 per cent peptone solution were tested at various stages of growth for indol, skatol and tryptophane. None was indicated in any test with the para-dimethylamidobenzaldehyde-HCl test; but a positive test for tryptophane was obtained in one culture with the more delicate vanillin— H_2SO_4 test.

Several tests for the presence of urea and for glycerin in broth and in egg albumen cultures gave negative results.

When the filtrates from peptone or egg albumen cultures were slowly evaporated at 50°C ., a small amount of crystalline precipitate was found; but, on account of the small amount available and the difficulties of purification, the exact physical constants of the crystalline body were not deter-

TABLE 4.—Results of qualitative analyses of liquid media after six weeks growth of *Sclerotium rolfssii*

Substances tested for	Results of tests in various media			
	Peptone	Peptone + 2 per cent dextrose	Broth + 2 per cent saccharose + 30 grams CaCO_3	3 per cent saccharose
Ammonia	+	+	+	0
Alcohol	0	0	0	0
Aldehyde	0	0	0	0
Acetone	0	0	0	0
Acetic acid	0	0	0	0
Formic acid	tr	tr	tr	tr
Butyric acid	0	0	0	0
Propionic acid	0	0	0	0
Cholic acid	0	0	0	0
Glycocholic acid	0	0	0	0
Taurocholic acid	0	0	0	0
Lactic acid	0	0	0	0
Oxalic acid	+	+	+	+
Succinic acid	+	+	+	+
Benzoic acid	0	0	0	0
Hippuric acid	0	0	0	0
Salicylic acid	0	0	0	0
Tannic acid	0	0	0	0
Gallie acid	0	0	0	0

mined. Chemical tests were positive for ammonia and oxalic acid and led to the inference that it was either the neutral or acid salt of ammonium oxalate.

Since qualitative examinations of cultures indicated that ammonia and oxalic acid were formed in considerable quantity from peptone and other media, it seemed desirable to learn whether or not the quantities formed were sufficient to produce the reaction changes which occurred.

For quantitative determination of oxalic acid the various media were prepared in 1-liter quantities in 2-liter flasks, autoclaved, inoculated with a single sclerotium from the pepper strain of the fungus, and incubated 31 days at 25° to 27° C. The mycelial mat was then removed, washed several times with distilled water, placed on a watch glass, dried 24 to 48 hours in

TABLE 5.—Amounts of oxalic acid found in 1-liter quantities of the media indicated, 31 days after inoculation with *Sclerotium rolfsii*

Medium	Mycelium, mgs.	Calcium oxalate, mgs.	Equivalent oxalic acid, mgs.
Beef-extract peptone broth	676	930	802
Beef-extract peptone broth + 2% saccharose	7575	1885	1625
1% peptone	469	845	728
2% do	705	2635	2271
3% do	760	550	474
4% do	No growth		
1% do + 2% saccharose	4352	2520	2172
1% do + 4% do	7895	5105	4401
1% do + 2% do	2740	1470	1267
2% do + 2% do	5340	4645	4004
4% do + 2% do	8620	7295	6288
1% do + 2% dextrose	5675	2000	1724
1% do + 2% maltose	7505	3045	2625
1% do + 2% corn starch	6277	2600	2241
1% do + 2% mannite	1025	850	733
1% do + 2% levulose	4750	1520	1310
1% do + 2% lactose	891	620	534
1% do + 2% glycerin	1580	1000	862
1% do + 2% citric acid	1050	10	9
1% do + 2% potassium citrate	No growth		
1% do + 2% calcium citrate	No growth		
2% calcium citrate + 10 cc. N/1 HCl	955	320	276
Czapek's solution	765	345	297
Decoction from 391.5 gms. string beans	2313	2180	1879
391.5 gms. string beans after extraction	Not determined	2530	2181

an oven at 45° C. and for another 24 hours at room temperature, and then weighed.

The culture fluid and washings from the mycelium were made slightly alkaline with ammonia, a concentrated solution of calcium chloride added, and allowed to stand 24 hours at 45° C. The supernatant liquid was then decanted off, the precipitate treated with strong acetic acid, filtered, washed with distilled water, re-dissolved from the filter with hydrochloric acid and again precipitated by neutralizing with ammonia. After standing 24 hours, the precipitate was collected on weighed filter paper, again washed with strong acetic acid and several times with distilled water, air dried in the same manner as the mycelium, and weighed. The results are shown in table 5. While this method of estimating total oxalic acid is not accurate, tests with peptone solutions containing known amounts of oxalic acid indicate that the error is comparatively small. Usually there is a slight loss of calcium oxalate through the filter paper.

For determination of ammonia production, culture solutions of broth and of broth plus 2 per cent saccharose were prepared and flasks, 100 cc. per flask, autoclaved, inoculated, and incubated six weeks at 25° C. Four strains of the fungus were used in each medium and one flask of each held as a check. The fungous mycelium was then filtered off, washed with distilled water, dried as in the previous experiment, and weighed. The filtrate was made up to 100 cc. with washings from the mycelium and placed in a Kjeldahl flask. Then 50 cc. were distilled over into 100 cc. of N/20 H₂SO₄ solution. Methyl red was then added as indicator and the solution titrated to neutrality with N/20 NaOH solution. The ammonia present was estimated from the difference in amount required to neutralize the check and the culture. The results are shown in table 6.

Another series of three flasks of each medium was run as a check on the previous test. Only the pepper and potato strains of the fungus were used and the third flask of each medium held as check, all being incubated 30 days at 25° C. The averages for the two strains were: broth, 147.5 mg. mycelium; 21.9 mg. NH₄; broth + 2% saccharose, 1,000 mg. mycelium; 9.8 mg. NH₄.

There appeared to be some disintegration of the mycelium at the end of the six-weeks' period. This may have affected both the weight of mycelium and the amount of ammonia in solution, doubtless increasing the latter by an amount formerly held within the hyphae.

The most striking feature of the results is seen on the two media, broth and broth + 2 per cent saccharose. Although the mycelium produced on saccharose broth is several times as much as that produced on broth, the amount of ammonia produced is only about half that produced on the latter medium.

TABLE 6.—Amounts of mycelium and of ammonia produced by *Sclerotium rolfsii* on 100 cc. quantities of broth and broth plus 2 per cent saccharose

Strain of <i>Sclerotium rolfsii</i>	Medium	Mycelium, mgs.	NH ₄ , mgs.	Medium	Mycelium, mgs.	NH ₄ , mgs.
Pepper	Broth	20	16.9	Broth + 2% saccharose	833	lost
Potato	do	65	20.1	Broth + 2% saccharose	900	11.9
Peach	do	Broth + 2% saccharose	1130	12.6
Cedrus	do	150	30.7	Broth + 2% saccharose	1120	10.2
Average		78.3	22.5		995.75	11.93

Reference to table 5 shows that oxalic acid production is much greater on saccharose broth than on plain broth. This increased oxalic acid and decreased ammonia production explains the much greater acidity produced in broth plus sugar and also suggests an explanation for the formation of both ammonia and oxalic acid in broth cultures. They are both probably waste products in the utilization of certain amino acids for growth energy and carbon nutrition in production of cell walls, etc. This theory is supported by the fact that ammonia production is greatly reduced by the presence of a readily available carbohydrate, such as dextrose; and oxalic acid production is almost entirely eliminated by the presence of citric acid, a readily available source of carbon which yields no oxalic acid.

In order to determine the proportions of ammonia and oxalic acid formed in broth cultures, the mycelium was filtered from a 500 cc. broth culture which had been incubated 30 days and the filtrate made up to 500 cc. with

TABLE 7. Actual and molecular-equivalent concentrations of ammonia and of oxalic acid in *Sclerotium rolfsii* culture solutions

Culture solution	pH	NH ₄ , mgs. per L.	Per cent. molecular concentration	Calcium oxalate, mgs. per L.	Oxalic acid, mgs. per L.	Per cent. molecular concentration
500 cc. broth, 30-days-old	3.8	227.00	1.200	2540	2189.5	1.737
Composite broth, 2-months-old	—	394.80	2.185	3255	2805.8	2.226
500 cc. broth + HCl, 30-days-old	3.8	346.04	1.915	935	806.0	0.623

washings from the mycelium on the filter paper. Two 100-cc. lots were used for ammonia determinations, and the other 300 cc. used for the determination of oxalic acid. For comparison, three 100-cc. broth cultures two-months-old in which the mycelium had noticeably disintegrated were filtered together, and the filtrate made up to 300 cc. Of this one 100-cc. lot was used for ammonia determination and the other 200 cc. for oxalic acid determination. Also a 30-day-old culture on 500 cc. of broth plus 10 cc. N/1 hydrochloric acid was included in order to learn how the free hydrochloric acid might be eliminated by the fungous growth. Two 100-cc. portions of the filtrate were used for ammonia determination, 200 cc. for oxalic acid and the other 100 cc. for determination of chlorides in solution.

In the latter, the chlorides were determined volumetrically by the Volhard-Harvey method [see Hawk (7), p. 577]. All of the chlorine added was found in solution, showing that it is not absorbed and held in combinations inside the mycelium, a theory once suggested as an explanation of its lack of effect on the end-point reaction of broth cultures.

The results of the ammonia and oxalic acid determinations, calculated for 1-liter portions, are shown in table 7. In the filtrate from both cultures on standard beef-extract broth, ammonia and oxalic acid were found in almost equimolecular concentrations. This was especially true in the old cultures where the solutes formerly held in the fungous hyphae had presumably passed into the culture solution.

In order to compare H-ion concentrations in acid ammonium oxalate solutions in concentrations equivalent to those formed in culture solutions, M/10 solutions of ammonium oxalate and of oxalic acid were prepared, and 500 cc. of each solution poured together into a flask. The mixture was then corked and set in a warm dark place for 10 days to allow chemical equilibrium to be reached. The solution was then shaken thoroughly and portions diluted to equal concentrations with distilled water and with beef-extract broth. The pH values of each dilution (colorimetric determination) are shown in table 8.

Although the concentration of peptone and beef-extract was considerably lowered by the addition of large quantities of oxalate solution, yet it

TABLE 8.—*Hydrogen-ion concentrations in various concentrations of acid ammonium oxalate in water and in broth*

	M/20	M/50	M/100	M/200	M/500	M/1,000	M/5,000	M/10,000
pH in H ₂ O	3.0	3.05	3.1	3.3	3.50	3.7	4.0	4.1
pH in broth	3.4	3.90	4.3	4.5	4.55	4.6	4.7	4.8

showed considerable buffer action. The diluted solutions were probably comparable to the solution left after growth of the fungus. In broth, acid ammonium oxalate produces an H-ion concentration near pH 4.0 over a wide range of concentrations comparable with concentrations produced by the fungus in broth cultures.

Further work will be necessary to explain definitely why ammonia and oxalic acid are formed in equimolecular proportions. The most likely hypothesis is that they are both formed as waste products in the utilization of the two mon-amino di-basic acids, glutamic and aspartic acid, for carbon nutrition. In the presence of free hydrochloric acid the proportion of ammonia to oxalic acid is much higher. Possibly the free hydrochloric acid makes other amino acids more easily decomposed by the fungus. On the other hand the reduced amount of oxalic acid may be due to the rapid decomposition of free acid in solution, but in this case we must assume a stimulative effect of hydrochloric acid to account for the greater ammonia production. If the former hypothesis is accepted, we need assume no effort on the part of the fungus to regulate the H-ion concentration. The end-point reaction is produced by the waste products from the utilization of the most readily available source of carbon.

TOXICITY OF CULTURE SOLUTIONS, OF OXALIC ACID, AND OF OXALATES

For the purpose of testing the toxicity of culture solutions in which *Sclerotium rolsii* had grown, seed of various plants were surface-sterilized and placed on the surface of sterile tap water agar (1½ per cent agar) in large test tubes. As the seed germinated, the roots pushed down into the agar and the plumule was raised sufficiently to allow a quantity of solution to be poured around the stem without coming into direct contact with the younger growing parts.

In the first test a month-old culture on 100 cc. of broth plus ½ per cent dextrose was filtered through a sterile filter into a sterile flask. The filtrate was then divided into two approximately equal portions. One portion was boiled for one minute and then allowed to cool to room temperature. The unboiled solution was poured about the base of four tomato and seven peanut plants growing in tubes as described previously. At the same time the boiled solution was poured about a similar number of seedlings. The same number received a sterile broth of the same age.

After 48 hours the stems of all the tomato plants in both boiled and unboiled culture filtrate were girdled, and the plants had collapsed. There were many small lesions on the peanut stems, but none of the plants was dead. The leaves of all plants were killed wherever touched by either of the culture filtrates. Neither tomato nor peanut plants showed any evi-

dence of injury from contact with the sterile broth. In the peanut seedlings the epidermis at the base of the stem was rather heavily cutinized and

TABLE 9.—*Toxicity of filtrates from Sclerotium rolfseii cultures on various nutrient solutions to seedlings of pepper, tomato, and soybean*

Culture filtrates	pH	Plants used	No.	No. plants injured		
				After 18 hours	After 48 hours	After 7 days
Broth	4.0	pepper	4	0	0	0
		tomato	2	0	0	0
Check — sterile broth	6.8	soybean	8	0	0	0
Broth + 2% dextrose	2.6	soybean	10	6, 1 dead	6, 1 dead	6, 1 dead
		tomato	5	5	5 dead	5 dead
		pepper	8	0	4 dead	4 dead
Broth + 2% dextrose boiled	2.8	soybean	12	4	4	6, 1 dead
		tomato	2	2	2 dead	2 dead
		pepper	5	0	3 dead	3 dead
1% peptone + 2% saccharose	2.3	soybean	4	2	3	3 ^a , 1 dead
		tomato	12	8	12 dead	12 dead
1% peptone + 2% saccharose, boiled	2.4	soybean	4	0	3	3 ^a , 1 dead
		tomato	9	6	9 dead	9 dead
1% peptone + 2% saccharose + CaCO ₃	3.0	soybean	5	0	1	3 ^a , 2 dead
		tomato	11	0	3	11 dead
1% peptone + 2% lactose (30-day culture)	4.1	soybean	4	0	0	0
		tomato	14	0	0	0
2% lactose in distilled H ₂ O	2.7	tomato	14	0	0	3 dead
3% saccharose in distilled H ₂ O	2.2	soybean	4	2	4
		tomato	8	5	3 ^a , 2 dead
3% saccharose in distilled H ₂ O, boiled	2.2	soybean	9	4	9
		tomato	5	2	3 ^a , 5 dead
3% saccharose in distilled H ₂ O, sterile	6.5	soybean	2	0	0	0
		tomato	6	0	0	0
3% saccharose in tap water	2.1	soybean	3	3	3	3
		tomato	7	7	7	1 ^a , 6 dead
3% saccharose in tap water, boiled	2.1	soybean	4	4	4	4
		tomato	9	9	9	9 dead

^a These plants were girdled.

contained few or no stomata. Such stems are comparatively impervious to solutions; hence peanut seedlings were not used in subsequent tests.

The results of the test indicated at once that some thermo-stable toxic substance capable of killing the tissue of tomato and peanut stems was present in the filtrate from dextrose broth culture, but was not present in sterile broth.

In order to gain some further insight as to the nature of the toxic substance, a large number of seedlings of tomato, pepper and soybean were grown in tubes of sterile tap water agar and in some cases on distilled water agar, and the toxicity of the filtrates from cultures on various media was tested. A summary of the results is shown in table 9.

The first evidence of injury from the culture solution filtrates was a bleaching of the epidermis in direct contact with the solutions, followed after a few hours by sunken lesions or complete girdling of the stems. Where the stem tissue was killed throughout, the seedling dropped down of its own weight. The plants receiving sterile culture solutions not only were not injured but after a few days started more vigorous growth than check plants. This was especially noticeable in tubes receiving sugar solutions.

No noticeable injury was produced by any culture solution less acid than pH 3.9, and the severity of the injury appeared to increase directly with increase in acidity. The toxicity is probably due solely to the hydrogen ions. The filtrate from a culture on cane sugar in water was very toxic although it contained less than one-fifth as much oxalic acid as the non-toxic filtrate from broth cultures.

Preliminary tests with solutions of oxalic acid showed this acid to be very toxic, producing lesions on the stems of seedlings indistinguishable from those produced by filtrates from *S. rolfii* cultures (Fig. 4). In order to determine whether or not it would be injurious in solutions comparable with the filtrate from a pure sugar solution culture, a series of very dilute solutions were tested on seedlings. All solutions had produced some injury at the end of 24 hours. In 1-10,000 solutions the injury was very slight but was more evident at the end of 48 hours. At the same time a 1 per cent solution of ammonium oxalate had produced no injury. By the end of 48 hours, however, molds had begun to develop on some of the solutions, and in further tests records were taken during the first 24 hours only.

As cutinized tissues are not readily penetrated by solutions and do not show slight injuries readily, the toxicity of oxalic acid, ammonium oxalate and urea oxalate was compared on disks of red beet root tissue. A longitudinal cylinder, 1.5 centimeters in diameter, was cut from a beet root with a sharp cork borer and was then cut into disks one-half to one millimeter

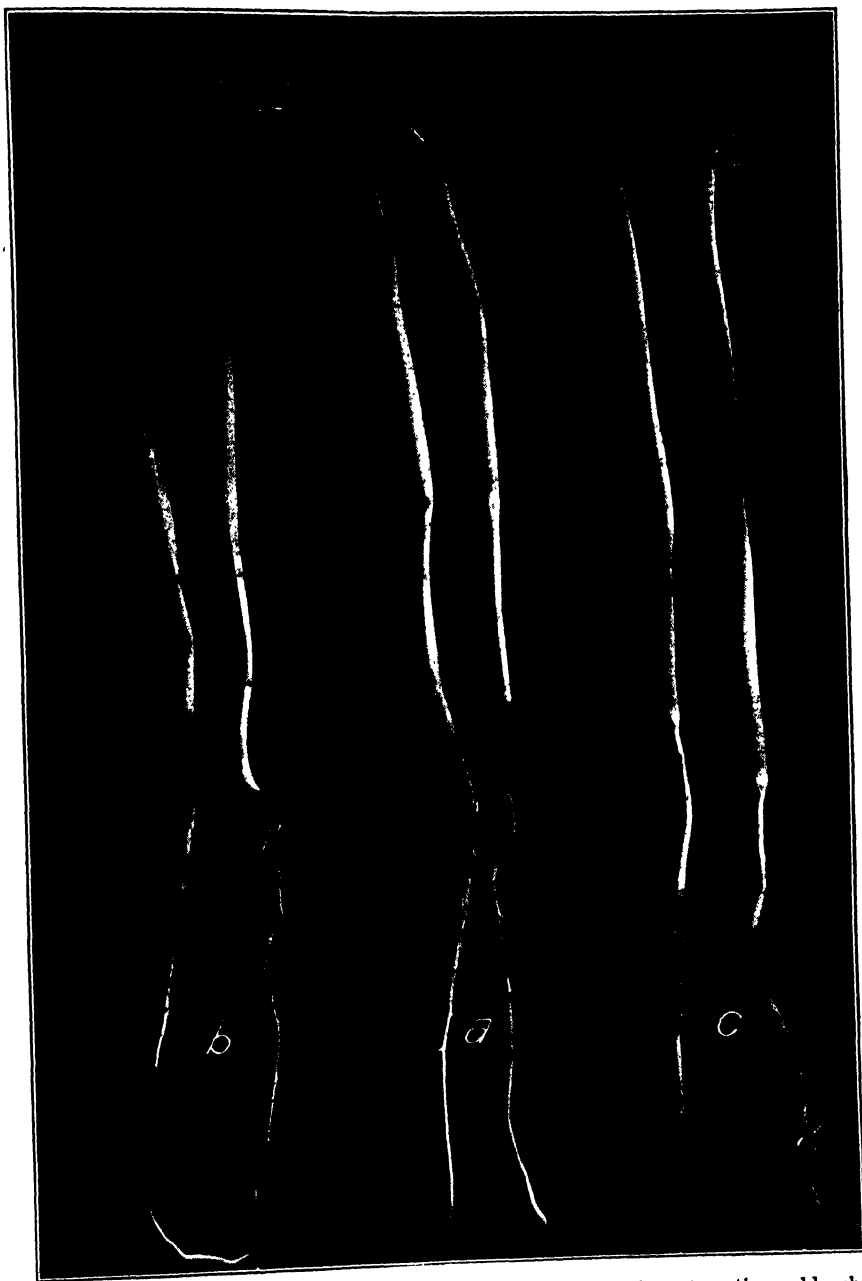


FIG. 4. Soybean seedlings from agar tube cultures, showing stem tissue bleached and collapsed after 72 hours contact with toxic solutions: (a) filtrate from 13-day-old culture of *S. rolfii* on broth plus 3 per cent saccharose, (b) 0.5 per cent solution of oxalic acid in sterile broth, (c) 0.1 per cent solution of oxalic acid in distilled water. Natural size.

thick. The disks were washed several times in distilled water and distributed five to each solution in culture dishes containing 30 cc. of solution. In non-toxic, dilute solutions the disks of beet root tissue became more turgid



FIG. 5. Pepper stem showing mycelium of *S. rolfsii* on surface (natural infection).

than at the beginning and retained the red pigment. In lethal solutions the pigment diffused into the liquid and the disks became flabby, as could

TABLE 10.—*Toxicity to plant stems and tissues of oxalic acid, ammonium oxalate, and urea oxalate as indicated at the end of 24 hours*

Oxalic Acid					
Plants	No. in each solution	Concentrations			
		1-1000	1-2000	1-5000	1-10,000
Tomato	6	dead	dead	injured	uninjured
Pepper	2	do	girdled	girdled	do
Cotton	6	do	dead	dead	injured
Beet root disks	5	do	do	do	partly bleached

Ammonium Oxalate							
Plants	No. in each solution	Concentrations					
		3-100	2-100	1-100	1-200	1-500	1-1000
Tomato	6	uninjured	uninjured	uninjured	uninjured
Beet root disks	5	uninjured	uninjured	do	do	do	do

Urea Oxalate							
Plants	No. in each solution	Concentrations					
		1-200	1-500	1-1000	1-2000	1-5000	1-10,000
Tomato	6	dead	dead	injured	uninjured	uninjured	uninjured
Beet root disks	5	do	do	dead	dead	partly bleached	do

TABLE 11.—*Toxicity of acid ammonium oxalate to beet root disks*

Solvent	Concentrations						
	M/20	M/50	M/100	M/200	M/500	M/1000	M/10,000
H ₂ O	dead	dead	dead	partly dead	slightly bleached	uninjured	uninjured
Beef-extract broth	do	do	slight injury	uninjured	uninjured	do	do

be seen when they were lifted by one edge. Near the limit of lethal dilution the thinner portions of the disks were bleached and dead, although cells in the thick portions remained alive. The results at the end of 24 hours are shown in table 10.

Because of the difficulty of obtaining the crystallized salt of mon-ammonium oxalate free of oxalic acid and of the double salt the equi-



FIG. 6. Peach seedlings girdled by *S. rolfii*.

molecular mixture of the acid and the double salt previously mentioned was used in toxicity tests. The results of the test are shown in table 11.

Comparison of the water solution with the results in table 10 shows that the acid ammonium oxalate is about equally toxic with urea oxalate and approximately half as toxic as pure oxalic acid. In broth the toxicity is greatly reduced, corresponding with the reduction in ionization in this solvent. This indicates again that toxicity is due to the hydrogen-ions in solution. This is in accord with the conclusions of Kahlenberg and True

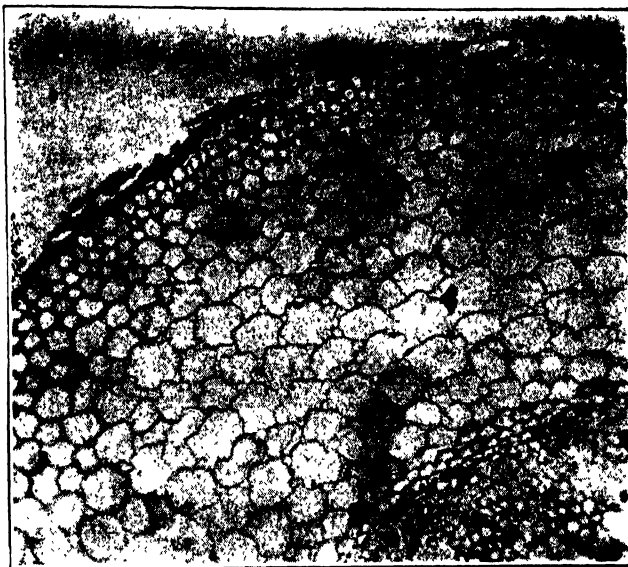


FIG. 7. Photomicrograph, portion of cross section of soybean stem in early stage of infection by *S. rolfsii*, and showing mycelium with holdfasts clinging to surface.

(8) in their study of the toxicity of oxalic and other organic acids to the roots of *Lupinus albus*, of Muth (12) in regard to the leaves and flowers of grapes and other seed plants, and of Uppal (18) concerning the spores of *Phytophthora colocasiae*. Oscar Loew (10), however, working principally with algae and also reviewing the work of Migula (11) on algae, reached the conclusion that the oxalic radical in solutions of oxalic acid and of oxalates has specific toxicity due to the precipitation of calcium from organic combinations in the nucleus.

Doubtless there is considerable variation between the various groups of plants in regard to the use of calcium and in regard to the toxic action of oxalic acid and of oxalates; but in the case of fungi and the seed plants so far tested the toxicity appears to be due to the hydrogen-ions in solution.

RELATION OF *SCLEROTIUM ROLFSSII* TO HOST TISSUE

In the field, plants killed by *Sclerotium rolfssii* are characterized by a considerable mat of mycelium over the surface of the stem near the surface of the soil (Figs. 5 and 6). Underneath this mycelium the bark is dead and decayed, but the central cylinder of mature plants is not decayed and such plants remain erect after death.

In order to study the relation of the mycelium to the host tissues, tomato and soybean seedlings were grown in tubes of sterile tap water agar. After the seedlings were well started, a single fungous sclerotium was dropped onto the surface of the agar at some distance from the seedling. A thin cobwebby growth of mycelium was produced over the surface of the agar. When it came into contact with the stem of the seedling, a thick white mat was formed over the base of the stem, but the plant was not killed until two to three days later. The stems thus attacked were examined microscopically by peeling off the epidermis and mounting in water. Some of the stems in various stages of attack were killed in chrom-acetic acid, embedded in paraffin, sectioned, and stained with haematoxylin.

The fungous mycelium clings to the stems by means of peculiar hold-fasts, thickened ends of hyphae which are more or less flattened on the

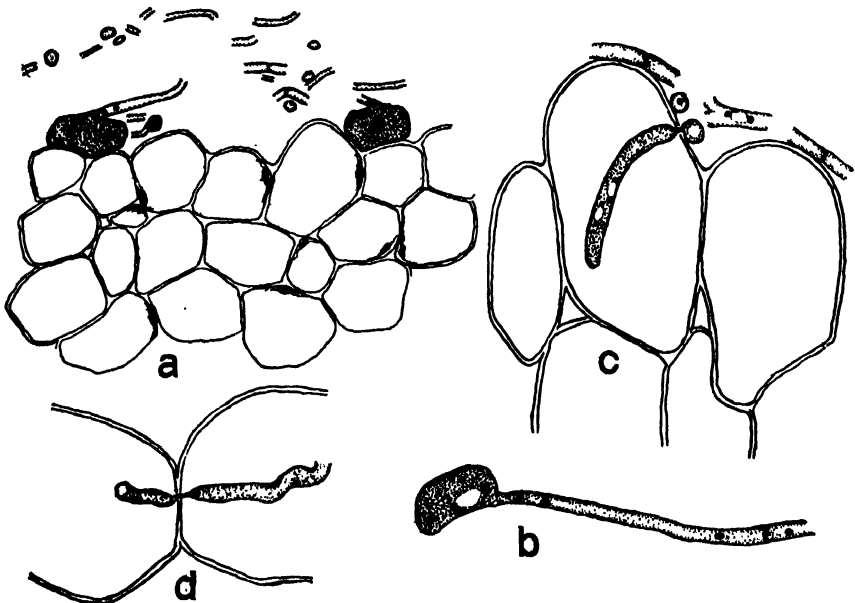


FIG. 8. (a) Section from surface of soybean stem in early stage of infection by *S. rolfssii*, showing relation of holdfasts and hyphae to epidermal cells. Both epidermal and parenchyma cells shown are already dead. (b) A single holdfast disengaged and more highly magnified. (c) Penetration of epidermal cell by hyphal branch. (d) Penetration of parenchyma cell wall by hypha.

Drawn with aid of camera-lucida. a, $\times 525$; b, c, and d, $\times 1750$.

surface in contact with the epidermal cells of the host (Figs. 7 and 8, a and b). The epidermal cells and the underlying parenchyma to a depth of two to four cells are killed before there is any evidence of hyphal penetration. Later the hyphae penetrate the dead cells and spread throughout the soft parenchymatous tissue. When passing through the cell walls the hyphae are much constricted and pass through very small openings (Fig. 8, c). This passage appears to be produced mechanically. In the initial penetration into the epidermal cells the outer wall is often crushed. Also there is little evidence of the dissolving action of enzymes even on the walls of parenchyma. Penetration of lignified tissues is very slow.

The epidermis with attached fungous hyphae was stripped from the base of some of the stems in early stages of infection and tested for the presence of oxalic acid by mounting in a weak calcium chloride solution, by Patschovsky's (13) ferrous-sulphate-acetic acid method and by Plahl's (15) silver nitrate-nitric acid method. All three gave crystals of oxalates in the epidermal cells, in the fungous hyphae, and on the surface of the hyphae.

In another experiment, cured sweet-potato roots were washed and placed in a moist chamber. After several sprouts appeared, sclerotia from the pepper strain of the fungus were placed on the surface of the root. The hyphae spread over the root and attacked the stems of the very young sprouts directly, just as they did on tomato and soybean seedlings. On older sprouts, however, not even the mycelial mat over the surface was formed. The hyphae in some cases climbed over the surface of these older sprouts until they came into contact with a young root or leaf, which they entered and killed, and then killed the stem of the sprout.

DISCUSSION

There is nothing especially unique in the temperature relations of *S. rolfssii* so far as the minimum and maximum temperatures for growth are concerned. The fact that vegetative growth is killed by even slight freezing may be of some significance in limiting the region in which the fungus can survive the winter. The dormant sclerotia can survive at least -10° C. for a short time, but sclerotia which have started growth are killed as readily as the mycelium. The very high optimum growth temperature, 30° to 35° C., and the fact that the fungus grows very poorly at temperatures below 20° C. are of greater significance. The latter is near the mean summer temperature in the cooler sections of the country. When considered in conjunction with other data reported in the present article, it leads to the conclusion that temperature relation is the principal factor in limiting the geographical range of the fungus.

The relation of the fungus to the reaction of the substratum can have little significance in relation to its distribution. Few of our arable soils are sufficiently alkaline to prevent growth of the fungus, especially where undecayed organic matter is present in the soil. In field experiments which may be reported in a later paper, great difficulty has been found in maintaining a reaction above pH 8.0 throughout a single season. In one test, where the soil had received a heavy application of cotton seed meal, hydrated lime was applied at various rates up to five tons per acre; yet at the end of four months the reaction of the plats receiving the heaviest applications of lime was barely above pH 7.0 and *S. rolfsii* was vigorously attacking plants in these plats. It seems, therefore, that maintaining an alkaline reaction of the soil as a means of protecting plants from attacks of this fungus is hardly feasible. Other methods of control are now being investigated.

Apparently, the principal function of sclerotia is to tide over periods of low food supply and unfavorable temperatures, and the length of the period of dormancy is governed entirely by these two factors. If transferred to suitable nutrients and favorable temperature at any period of development they begin to grow immediately. They have been observed to germinate and renew growth in old cultures in which the mycelium had largely disintegrated. Extracts of mycelium and even autoclaved cultures make very good media for growth, all of which indicates that dormancy is not induced by the presence of any toxic substance. The influence of a suitable nutrient on germination of sclerotia is further indicated by their failure to germinate on tap water and on water containing many organic substances not toxic to the fungus. We must infer that such substances are not sufficient to stimulate germination.

Ammonia and oxalic acid in the quantities ordinarily found in cultures are not toxic to the fungus.

The fact that certain fungi and bacteria are capable of producing oxalic acid has been known to botanists for more than 50 years. The early work and observations on the subject are summarized in several publications of Wehmer (19, 20 and 21), who made the earliest comprehensive investigations on the production of this acid by fungi growing on various classes of nutrients. Discussions of the results of subsequent investigators may be found in the more recent publications of Wehmer (22), of Currie and Thom (4), of Raistrick and Clark (16), of Gotoh (6) and of Cooley (3).

Knowing the toxic nature of oxalic acid, several botanists have suspected its responsibility for the death of host tissue previous to or during its penetration by certain species of parasitic fungi.

Many references to the work of De Bary on *Sclerotinia libertiana* Fekl. attribute to him the conclusion that this fungus produces an enzyme which kills the host tissue. [See Klebahn (9), p. 399, also Cooley (3), p. 293.]

With this conclusion in mind, the observation that when milk or egg albumen solutions were inoculated with *S. rolfssii*, the proteids were first coagulated and then slowly digested during growth of the fungus, immediately suggested that a protein coagulating enzyme was produced. Several attempts were made to demonstrate the presence of such an enzyme in culture solutions and in the mycelium but always with negative results, and the conclusion was finally reached that the coagulation of these substances in cultures was due entirely to acidity.

Upon close study of De Bary's (2) original publication, it appears that his final conclusion on the subject is indicated in the statement (p. 420) that an enzyme destroys the cell walls and may or may not be responsible for the death of the protoplasts, as organic acids (such as oxalic) and their salts are known to be toxic to plant protoplasts.

Smith (17), in his study of the parasitism of *Botrytis cinerea* on lettuce plants, found that the cells were killed, before penetration of the fungous hyphae, by a thermostable toxic substance, and expressed the opinion that this toxic substance was oxalic acid.

Peltier (14), studying the parasitism (supposedly) of the same fungus on lettuce and pepper plants, found a thermostable toxic substance in extracts from the fungous mycelium but failed to find oxalic acid in the extract. He expressed the opinion that this toxic substance was some organic acid but not oxalic.

Cooley (3) found that *Sclerotinia cinerea* produced considerable quantities of oxalic acid from plum and peach juice and from peach fruits but expressed no definite opinion as to the significance of the results.

Whatever the case may be with regard to other fungi, the evidence seems quite conclusive that oxalic acid is responsible for the death of the host tissue in the plants attacked by *Sclerotium rolfssii*. (1) Plants were killed by the filtrate from cultures on pure sugar solutions where oxalic acid appears to be the only metabolic product formed in any significant quantity. (2) In the filtrate from cultures on other media the toxicity increased directly with the increase in free acid. (3) The injuries were similar to injuries produced by pure oxalic acid solutions. (4) Considerable quantities of oxalic acid or soluble oxalates were found in the dead host cells, even before entrance of the fungous hyphae; whereas none was found in healthy cells of the same plants.

Accepting this conclusion, we can easily understand the very wide range of host species attacked by this fungus. Apparently, any plant with an epidermis easily permeable to oxalic acid solutions is susceptible to attack. The fungus does not penetrate living tissues, and there is no occasion for the development of special adaptations between parasite and host.

SUMMARY AND CONCLUSIONS

1. The minimum temperature for growth of *Sclerotium rolfsii* is near 8° C. The absolute maximum is near 40° C., but 37° C. appears to be the maximum for continuous normal growth. The optimum growth temperature lies between 30° and 35° C. Growth is poor below 20° C.

2. Temperature relations appear to be the limiting factor in determining the geographical range of the fungus.

✓ 3. The fungus does not appear to utilize inorganic nitrogen readily when supplied either as nitrates or as ammonium salts.

4. There is a slight variation in cultures of the fungus from different sources in their relation to the reaction of the substratum; but on beef-extract-peptone broth none of the cultures grew when the reaction was more alkaline than pH 8.3 or more acid than pH 1.4.

5. The addition of 1 per cent NaCl to broth shortened the growth range of the fungus on both the alkaline and the acid side of neutrality.

6. The addition of 2 per cent saccharose to broth extended the growth range on the alkaline side but shortened it on the acid side.

7. On broth and broth plus NaCl, the fungus, during growth, changes the reaction of the medium toward a fairly definite end-point near pH 4.0.

8. On broth plus cane sugar the reaction change is always toward the acid side until the sugar is used up.

9. This reaction change is due to the production of ammonia and oxalic acid from peptone and of oxalic acid from saccharose.

10. In broth cultures ammonia and oxalic acid accumulate in nearly equimolecular proportions. Apparently both are waste products in the utilization of certain amino acids as a source of energy and carbohydrate nourishment.

11. Oxalic acid is also produced from a number of other carbohydrates and from various plant decoctions. None is produced from citric or tartaric acids.

12. Filtrates from cultures of the fungus growing on certain nutrients are toxic, both before and after boiling, to seedlings of tomato, pepper and soybean.

13. The injuries produced by the filtrates are very similar to those produced by oxalic acid solutions.

14. The toxicity increases with the increase of H-ion concentration and the increase in concentration of free oxalic acid in the filtrate.

15. The toxicity of the filtrates and of oxalic acid solutions to the plants tested appears to be due entirely to the hydrogen-ions in solution.

16. In parasitic attacks on plants the fungus forms a considerable mat over the attacked portion, clinging to the epidermis by means of holdfasts.

The underlying cells are killed before the fungous hyphae enter the host tissue.

17. Considerable quantities of oxalic acid or soluble oxalates are found in these dead cells, while none is found in healthy cells of the same plants.

18. The evidence appears to be conclusive that the death of these cells is due to the toxic action of oxalic acid and that the oxalic acid is secreted by the fungous hyphae.

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STUDIES ON THE SCUTELLUM ROT DISEASE OF CORN¹

BENJAMIN KOEHLER

INTRODUCTION

Scutellum rot is a very common disease of dent corn (*Zea mays indentata*). It also occurs in *Zea mays saccharata*, *Zea mays amylacea*, *Zea mays indurata*, and perhaps in other subspecies.

Corn kernels affected with this disease were illustrated by Hoffer and Holbert (6) in 1918, but the disease was not clearly defined or distinguished from other seedling rot diseases. Adams and Russell (1) clearly described scutellum rot as caused by *Rhizopus nigricans*. Holbert *et al.* (8) published extensive data on the field performance of plants grown from seed susceptible to this disease and named the malady "scutellum rot."

To judge from results of tests of seed corn for germination and freedom from disease conducted by several commercial seed-corn testing establishments, the disease is very common in all parts of the corn belt from which corn had been obtained. In germinating the corn exhibited in the Fifth Utility Corn Show held at Urbana, Illinois, 1925, records were made of all the corn diseases that appeared on the germinator. It was found by Koehler and Pettinger (10) that about 21 per cent of the kernels from the ear corn exhibited in the show developed a scutellum rot on the germinator. This show corn should represent, however, the best seed corn of the state. Most of the seed corn would doubtless have shown a higher percentage of infection. Many lots of seed corn obtained from the central portion of the corn belt and tested by the writer during the past six years were very nearly 100 per cent susceptible to this disease when tested on the limestone-sawdust germinator. This germinator has been described by Holbert *et al.* (8). Seed corn obtained from the Philippine Islands and from Argentina, South America, also developed this disease on the germinator.

Control measures for this and other seed-borne corn diseases have been described by Hoffer and Holbert (6), Holbert and Hoffer (7), and Holbert *et al.* (8). The recommendations consist essentially of selecting ears from sturdy, healthy stalks in the field, proper curing, culling down to a type of ear that has been found to be most disease resistant, then testing the selected

¹ Contribution from the Department of Plant Pathology, University of Wisconsin, and the Department of Agronomy, University of Illinois. Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin.

ears on a good germinator, and discarding any that show the presence of disease.

NATURE OF THE DISEASE

Typical scutellum rot usually is not caused by a parasite carried within the corn kernel, but is caused by infection from without after the kernel begins to germinate and the seed coat has been ruptured. When germinated on a limestone-sawdust germinator at about 27° C. (80° F.), most of the radicles break through the pericarp during the second day, but the plumules appear a little later, usually not until the third day. Following the rupture of the pericarp of susceptible seed, certain molds are able to enter and cause scutellum rot. On the sixth or seventh day at this temperature the rots can be seen very easily when the kernels are bisected. Kernels that fail to sprout seldom develop scutellum rot of the type herein described.

Four rows of sprouted kernels representing four different ears are shown in figure 1. The first and third rows are resistant to scutellum rot; the other rows show *Rhizopus* infection in abundance. A prolific growth of *Rhizopus* on a kernel is a fair indication of scutellum rot. To make a more accurate observation, however, the kernels must be bisected and examined closely. Figure 2 shows longitudinal sections of a good kernel (A), and kernels having various types of scutellum rot (B-F). In B the epithelium of the scutellum has rotted and appears dark in the illustration. C is a little more advanced stage of the same type, while D is still further advanced, the rot involving all of the cortical tissue of the scutellum. Rots of the above forms are typical for infections by species of *Rhizopus*. These organisms also ramify throughout the endosperm but do not cause a discoloration of this tissue.

Another type of rot sometimes produced by *Rhizopus*, but perhaps more often by other organisms, is shown in figure 2, E, the points of infection being at the bases of the lateral roots. When this type of infection develops early, some of the root initials may be destroyed, hence those particular roots fail to develop.

A rot at the base of the scutellum, as shown in figure 2, F, is characteristic for *Aspergillus* infection but may also be caused by some other organisms. Entrance of the organism apparently was effected through the tip end of the kernel and was not dependent on the rupture of the pericarp. It has been shown by Manns and Adams (14) that the dormant mycelium of *Aspergillus* spp. may be found beneath the cap at the tip of the kernels of dry corn. The writer has often found this type of rot after inoculating disease-free corn with species of *Aspergillus*. These latter organisms may, however, also cause the epithelial rots shown in B and C and the central rot

shown in E. From the condition shown in B, C, E, and F, the rot may extend to include the whole scutellum, as shown in D.

Scutellum rot infection usually results in a weakened condition of the seedlings from which the plants never fully recover. Two factors evidently cause this weakening. In the first place, the fungus destroys much of the enzyme secreting and absorbing tissue of the scutellum and separates the latter from its nutrient base. Sometimes the injury is limited to this region. In a more progressive form of the disease, the fungus enters and disorganizes the cortical tissue of the scutellum, and from there it invades the vascular tissue of the embryo. *Rhizopus* and *Aspergillus* have fre-



FIG. 1. Scutellum rot symptoms on the germinator. Each row of kernels represents an ear of corn. The first and third rows are free from mold. Considerable *Rhizopus* growth occurs in connection with the kernels of the other rows. The scutellum of such kernels usually is in a rotted condition.

quently been isolated from the mesocotyls of infected plants grown in soil, following seed inoculation with these organisms.

When seed susceptible to the disease is planted in experimental plots and its performance is compared with that of plants from disease-resistant seed, some of the features noticed are a reduced stand, weaker plants, and decreased yield. Data on 54 experiments given by Holbert *et al.* (8) over a period of seven years in different parts of Illinois show an average reduction in yield of 12.4 ± 0.66 bushels, or 17 per cent.

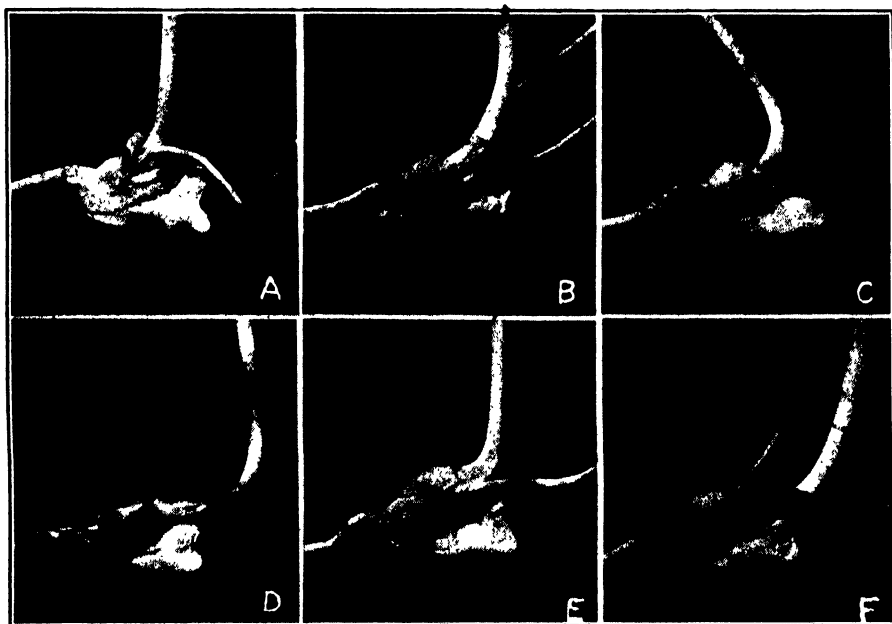


FIG. 2. Bisected kernels of germinated corn showing scutellum rot symptoms. A—healthy; B—epithelial rot of the scutellum; C—more advanced stage in which the rot has progressed to the cortical and vascular tissues; D—still farther advanced stage involving the whole scutellum; E—a rot at the base of the lateral roots; F—a rot of the base of the scutellum.

ORGANISMS RESPONSIBLE FOR SCUTELLUM ROT

Hoffer and Holbert (6) state that species of *Gibberella*, *Fusarium*, *Verticillium*, *Rhizopus*, and *Pseudomonas* cause diseases of corn plants, and in the same publication they illustrate typical cases of scutellum rot (Figs. 15–16). However, these authors do not mention which organisms are particularly responsible for the scutellum rot condition. Adams and Russell (1) found that *Rhizopus nigricans* is able to cause scutellum rot of corn, and they gave the pathological histology of infected kernels. Taubenhaus (15) found that the black and yellow molds of corn (*Aspergillus niger* and

A. flavus) had a detrimental effect on corn seedlings. However, he mentions only root rot as being due to these organisms and not scutellum rot. Manns and Adams (14) found that species of *Penicillium* and *Aspergillus* were limiting factors in germination. Holbert *et al.* (8) state that *Rhizopus* spp. are the most common molds causing scutellum rot on the germinator. They state further that corn subject to *Rhizopus* attack on the germinator is more susceptible to attacks of soil fungi and to injury from inoculation with other organisms than is corn that is resistant to scutellum rot on the germinator.

Pure Culture Inoculations

In order to determine whether *Rhizopus* species other than *nigricans* may cause scutellum rot, a number of species were secured for inoculation purposes. As some of these species are common in both soil and air, the tests had to be conducted under aseptic conditions. This necessitated the use of containers from which all living organisms could be excluded except those which were to take part in the experiment. A few other fungi which sometimes seem to cause scutellum rot were also included in these tests. It was shown by Lauritzen and Harter (11, 12) that different species of *Rhizopus* may behave in dissimilar manner at different temperatures. Therefore the tests were conducted under three different temperature conditions, namely, 16°, 22°, and 30° C., the first being near that of the soil temperature at corn planting time, the second representing ordinary room temperature, and the last being as high as that at which corn germinators are often run.

Source of Cultures.—Experiments were conducted with 12 species of *Rhizopus* which had been obtained from two sources. Cultures of *R. chinensis* Saito, *R. arrhizus* Fischer, *R. reflexus* Bainier, plus and minus strains, *R. microsporus* van Tiegh., plus and minus strains, and *R. pyriformis* marked "New sp. Eddy" were obtained from Dr. C. Dreschler. Cultures of *R. nodosus* Namysl., *R. nigricans* Ehrenb., *R. tritici* Saito, *R. maydis* Bruderl., *R. artocarpi* Racib., *R. oryzae* Went. and Pr. Geerlings, and *R. delemar* (Boid) Wehmer and Hanzawa were obtained through Dr. J. G. Dickson from Dr. L. L. Harter.

In addition to the above, a culture of *R. nodosus* marked "b," and cultures of *Aspergillus niger*, *A. flavus*, and *Penicillium* sp. were isolated from germinating corn kernels and pure lined for inoculation purposes by the writer.

Methods.—Inoculations were made on kernels from 10 ears of corn which, in a previous test on a limestone-sawdust germinator, had shown 60 to 80 per cent scutellum rot infection when inoculated with a spore suspension of *Rhizopus nodosus*. Culture dishes 100 mm. wide by 45 mm. deep were used in these tests. In preliminary trials some dishes were prepared with

soil, some with potato dextrose agar, and some with plain non-nutrient agar. When soil was used, it had to be sterilized on three successive days at 15 pounds pressure to insure sterile plates. When agar was used, one sterilization was sufficient.

In the preliminary trials in which the corn kernels were placed on sterile soil, nutrient agar, and plain water agar, respectively, and inoculated with *Rhizopus nodosus*, the resulting percentages of scutellum rot obtained were very similar. In the case of the soil and nutrient agar, a little of the mycelium with the accompanying spores was transferred to the center of the culture dish, but when plain water agar was used, it had to be placed directly on each kernel. This made the latter method a much more tedious one, with increased opportunity for contamination. On the nutrient agar the excessive fungous growth was objectionable. The tests made on soil were very satisfactory, although repeated sterilizations were necessary, excessive glass breakage occurred, and the soil etched the glass very badly during the sterilization process. A very satisfactory culture medium was finally prepared by using 1.5 per cent agar, and 1.0 per cent cornmeal. This was cooked in a steamer for one hour and then strained through fine cheese cloth. Inoculations were made in the center of the dish only, and the resulting fungous growth was not excessive but was comparable to that on soil.

Ten kernels of corn, one kernel from each of the 10 ears, were sterilized, washed, and placed in a circle, germ side up, in each dish. At the 16° temperature the corn was grown in the culture dishes for 28 days, at 22° C. for 10 days, and at 30° C. for 8 days.

Griffin low form beakers of 100 cc. capacity were used as containers in sterilizing and washing the corn. These were covered with aluminum dishes 65 mm. in diameter and 15 mm. deep.² (See figure 3.) One set of dry beakers with covers was sterilized by dry heat. A second set, comprising three times the number used in the first, was filled two-thirds full of water and autoclaved. The corn was introduced into the dry beakers after they had cooled. A 50 per cent alcohol solution was poured on the corn and then immediately drained off, and the beaker nearly filled with a 0.5 per

² These dishes are regular stock sold by dealers in scientific apparatus (Central Scientific Company, Catalog No. 3850). The Pyrex catalog quotes this size Griffin beaker only with the lip, but the manufacturers of Pyrex ware state that they will furnish them without lips if so specified in the order. These beakers do not corrode or break in the sterilizing process and withstand considerable rough handling. The Griffin low form is superior to the regular tall form beakers because they stand more securely, are easier to reach into with the forceps, and fit better with the aluminum covers. At first the tops and bottoms of 75 mm. petri dishes were used for covers, but the breakage on these in the autoclave was very high, especially when the vessels were stacked several deep. Furthermore, they were a little too large for a good fit on the beakers. The aluminum covers are very nearly the right size.

cent solution of Uspulun. The corn kernels were treated in this solution for one and one-half hours at 30° C. The above disinfectant was used in preference to the usual mercury bichloride when it was desired to germinate the corn. Uspulun, as well as some other compounds of similar nature, has very little, if any, detrimental effect on the vitality of corn kernels. When it was desired to surface-sterilize germinated kernels preparatory to making



FIG. 3. Apparatus used in surface sterilizing and washing plant tissue.

fungous isolations, a five-minute treatment with mercuric chloride 1:1000 was used. After treating the corn, the solution was poured off and the kernels poured into one of the beakers prepared with sterile water. This is a more rapid operation than picking up the corn kernels one at a time with forceps. After leaving the corn in the sterile water for 20 minutes, the operation was repeated except that this time the sterile water was poured onto the corn instead of dropping the corn into the sterile water. Three washings of 20 minutes each were given. For the last two washings, if desired, the sterile water could perhaps just as well be prepared in larger flasks plugged with cotton.

In order to keep the corn kernels from leaving the beakers when the liquids were poured off, a disk of perforated zinc was cut so as to pass easily into the beakers. A metal handle was fastened at right angles to the center of the disk (Fig. 3). This retainer was kept in a beaker of 95 per cent alcohol and was flamed before using.

All the transfers were made in a special transfer room which was kept as clean as possible. Practically no trouble with contamination was experienced.

Inoculations in Culture Dishes.—Data from inoculation trials with all the *Rhizopus* species, as well as with several other organisms, are given in table 1. As given in this table, rot type 1 refers to the epithelial rot illustrated in figure 2, B and C, and also to the more advanced stage of this rot progressing into the cortical tissue shown in D. Type 2 refers to the central rot at the base of the lateral roots shown in figure 2, E; type 3 refers to the rot at the tip end of the kernel (base of scutellum) shown in F. The *Rhizopus* species are arranged in descending order according to their effectiveness in producing scutellum rot. All the species used were more or less active in this respect. Five trials of ten kernels each were made with each species at the different temperatures. Several controls were included in each series and these always remained free from infection. Very

TABLE 1.—Percentages of scutellum rot at temperatures of 16, 22, and 30° C. when corn was inoculated with species of *Rhizopus*, *Aspergillus*, and *Penicillium*

Organisms	16° C. (28 days)			22° C. (10 days)			30° C. (8 days)			Total rot in per cent			
	Type ^a and percentage of rot			Type and percentage of rot			Type and percentage of rot						
	1	2	3	1	2	3	1	2	3	16° C.	22° C.	30° C.	Mean
<i>Rhizopus oryzae</i>	30	36	0	48	20	0	60	18	2	66	68	80	71.3
<i>R. nodosus</i>	48	12	2	32	22	0	66	14	0	62	54	80	65.3
<i>R. nodosus</i> (b)	32	18	0	28	14	2	58	14	0	50	44	72	55.8
<i>R. nigricans</i>	24	16	4	38	4	0	64	10	0	44	42	74	53.3
<i>R. tritici</i> ..	22	22	0	22	14	2	62	2	0	44	38	64	48.7
<i>R. chinensis</i> ..	36	24	2	12	16	4	36	10	0	62	32	46	46.7
<i>R. maydis</i>	34	16	2	24	16	0	42	6	0	52	40	48	46.7
<i>R. arrhizus</i>	32	18	0	32	12	2	34	8	0	50	46	42	46.0
<i>R. microsporus</i> (+) ..	8	12	4	12	22	2	26	18	0	24	36	44	34.7
<i>R. pyriformis</i>	22	14	2	14	10	0	24	10	0	38	24	34	32.0
<i>R. artocarp</i> i	16	28	0	16	4	0	24	0	0	44	20	24	29.3
<i>R. microsporus</i> (–) ...	8	16	4	12	6	0	28	8	2	28	18	38	28.0
<i>R. reflexus</i> (+) ..	22	18	2	8	6	4	16	8	0	42	18	24	28.0
<i>R. delemar</i>	14	12	0	8	20	0	10	10	0	26	28	20	24.7
<i>R. reflexus</i> (–) ..	12	4	2	12	6	0	10	4	0	18	18	14	16.7
<i>Aspergillus niger</i>	16	12	28	24	28	26	32	16	8	56	78	56	63.3
<i>A. flavus</i>	8	10	16	12	10	8	14	2	0	34	30	16	26.7
<i>Penicillium</i> sp.	10	16	2	6	22	6	8	12	0	28	34	20	27.3

^a 1 = type shown in Fig. 2, B, C, and D.
2 = type shown in Fig. 2, E.
3 = type shown in Fig. 2, F.

little trouble was experienced with contaminations. When a contamination was noted, the dish was discarded and the test repeated.

In this experiment (table 1), *Rhizopus oryzae*, *R. nodosus*, and *R. nigricans* were the three organisms most active in producing scutellum rot. *R. tritici*, *R. chinensis*, *R. maydis*, and *R. arrhizus* were about alike in pathogenicity, and come next in order. Plus and minus strains of *R. microsporus* and *R. reflexus*, respectively, were very much alike in their behavior. Both species were comparatively low in ability to produce scutellum rot.

All the organisms were effective at the three temperatures used. None of the temperatures was high enough to inhibit growth of any of the fungi. The first five organisms in table 1 were most active at 30° C., whereas some others, such as *R. chinensis*, *R. maydis*, and *R. artocarp*i, were most active at 16° C. *R. artocarp*i is the only species in this group that closely resembles *R. nigricans*. It has the same type of growth, the same limitations with respect to high temperature, and has large spores. The spores are apparently a trifle larger and more irregular than in *R. nigricans*. Each species produced 44 per cent infection at 16° C., but at 22 and 30° C. the latter was much more active than *R. artocarp*i.

It has been shown by Harter and Weimer (5) that physiological variations exist between different isolations of *R. nigricans*. The same is probably true of the other species of *Rhizopus*. The two cultures of *R. nodosus*, according to table 1, did not behave exactly alike. There is a probability, however, that this difference may be due to experimental error.

It should be noted that the rot of type 1 predominates in *Rhizopus* infection, while type 2 is a close second. Type 3 is comparatively rare. *Aspergillus* infection, on the other hand, readily causes all three types of rots, type 3 predominating at the low temperature used.

Harter, Weimer, and Lauritzen (4) used all these *Rhizopus* species with the exception of *R. pyriformis* in inoculation trials on sweet potatoes. They found all of them pathogenic with the exception of *R. chinensis* and *R. microsporus*. Lauritzen and Harter (11) came to the conclusion, however, that *R. nigricans* and *R. tritici* are the species primarily responsible for the decay of sweet potato known as soft rot, *R. nigricans* at temperatures between 6° and 20° C. and *R. tritici* at 30° C. and above, the two overlapping between 20° and 30° C.

Isolations from Rhizopus-infected Corn Kernels Tested on Germinator

It has been frequently stated that *Rhizopus nigricans* is the species which occurs most abundantly. References to fruit and vegetable rots due to attacks by this species are numerous. Perhaps the identifications have usually been correct. Harter *et al* (4), however, call attention to the common error of classifying all such molds as *Rhizopus nigricans*

Very likely many mistakes of this kind have been made. *R. nigricans* can be rather easily distinguished from other species because of its inability to grow at certain temperatures, and its large spore size. Only *R. artocarpi* falls in the same class and is hard to distinguish from *R. nigricans*. The chief difference observed by the writer in the cultures available, aside from slight spore differences, is that the vegetative portion of *R. artocarpi* grew less actively than that of *R. nigricans*.

In the central part of the corn belt, *Rhizopus* species are the most common cause of scutellum rot on the germinator. In order to determine which particular species occur most commonly, infected kernels showing the *Rhizopus* mold on the exterior, as in figure 1, were taken from the germinator at different times and from samples of corn obtained from widely separated places. *Aspergillus* spp. and other fungi sometimes cause scutellum rot on the germinator, but in this case only *Rhizopus*-infected kernels were selected. The plumule and roots were clipped to within about one-half inch from the kernel. Then the kernels were surface-sterilized for five minutes in mercuric chloride according to the method already described. After

TABLE 2.—*Fungi isolated from corn seedlings affected with scutellum rot*

Organisms isolated	No. of isolations from corn germinated on limestone-sawdust germinator for 7 days at 27° C. 132 seedlings	No. of isolations from corn germinated in virgin prairie soil for 30 days at 16° C. 61 seedlings
<i>Rhizopus tritici</i> type	63	...
<i>Rhizopus nodosus</i> type	60	6
<i>Rhizopus microsporus</i> type	2	...
<i>Rhizopus</i> spp. (undetermined)	7	...
<i>Mucor</i> spp.	24
<i>Pencillium</i> spp.	14
<i>Gibberella saubinetii</i>	3
<i>Fusarium moniliforme</i>	2
<i>Fusarium</i> spp.	12
<i>Aspergillus niger</i>	1
Genus undetermined	5

washing in sterile water, the kernels were opened with sterile instruments and a small piece of the infected tissue was placed on potato dextrose agar. From the resulting fungous growth, single spore cultures were made by the dilution plate method, using Beyerinck's agar. A total of 132 isolations were made, each from a separate kernel. The organisms were identified according to the descriptions given by Hanzawa (2, 3) and Lendener (13) and by comparison with the species in culture, the sources of which have already been given. The results are given in table 2.

The taxonomy of all the *Rhizopus* species is not as clear as one might wish, yet certain species or groups are well defined. All the cultures obtained in these isolations grew luxuriantly at 37° C. This excludes the possibility of any of them being *R. nigricans*, *R. artocarp*i, or *R. reflexus*. According to Weimer and Harter, their culture of *R. microsporus* did not grow at 37° C., but Drechsler's culture of the same species did grow at that temperature, and according to Lendener it should do so. All the cultures sporulated abundantly at room temperature. This eliminated the possibility of any of them being *R. chinensis*, *R. arrhizus*, or *R. maydis*.

Two small-spored forms isolated were identified as *R. microsporus* although the sporangial mass was a dark brown color; whereas the culture from Drechsler was more nearly dark gray on the same culture medium. Spores of all the other isolations were of medium size, and most of them could be assigned to one of two definite groups, one of which was classed as *R. tritici*, the other as *R. nodosus*. The former showed no zoning on potato dextrose agar slants, while the latter showed a distinct zone between the mycelial and sporangial masses, as shown in figure 4. When grown in flasks, it was also noted that the former did not grow as tall as the latter. In these characters as well as in all other aspects that were observed, the forms isolated were identical with the cultures of these species which were received from Dr. L. L. Harter. *R. delemar* and *R. oryzae* obtained from the same source are very similar to *R. tritici* and *R. nodosus* in many respects, but their mycelial mats in cultures have a finer texture. Typical nodes, however, were very rare in these cultures of *R. nodosus* on potato dextrose agar. In the many mounts of this fungus observed, the writer saw only two nodes resembling those figured by Lendener (13).

These data seem to indicate that *R. tritici* and *R. nodosus* are the most common organisms causing scutellum rot on the germinator. In the pure culture inoculations it was shown that *R. nigricans* is a very active causal agent of scutellum rot, but in 132 isolations of *Rhizopus* on the germinator it was not found. The writer does not wish to infer that it is never found in this connection, but it at least seems evident that in the central part of the corn belt it is not usually the predominating agent.

Isolations from Scutellum Rot-susceptible Kernels Grown in Soil

A number of ears of dent corn representing several varieties and sources were selected that averaged about 70 per cent susceptibility to scutellum rot on the germinator. A composite lot of 100 kernels from these ears was planted in virgin soil in a greenhouse where the temperature was maintained very close to 16° C. After 30 days the seedlings were taken from the ground and all soil carefully washed off. Isolations were then made in the manner previously described.

Of the 100 kernels planted, 61 showed scutellum rot infection. Although *Rhizopus* species are the principal cause of scutellum rot on the germinator, in this case only 6 kernels, or about 10 per cent (see table 2), were infected with *Rhizopus*. On the other hand, there were higher percentages of infection with *Mucor*, *Penicillium*, and *Fusarium*. Cultures of *Gibberella saubinetii* and *Aspergillus niger* were also isolated. All types of infection shown in figure 2 were found. In most cases the rot seems to have started in the epithelial region, just as it usually does on the germinator.

FACTORS ASSOCIATED WITH DISEASE RESISTANCE OR SUSCEPTIBILITY

Stage of Maturity

During the season of 1924, yellow dent corn of the utility type (10) was gathered in the milk, glaze, dent, mature, and husking stages by Mr. S. S. Carney.³ Germination tests of this corn for vigor and freedom from disease were made by the writer. The ears were dried in a well ventilated warm room. There were 70 ears in each group, and 10 kernels were tested from each ear. In the following season similar groups, with the exception of ears

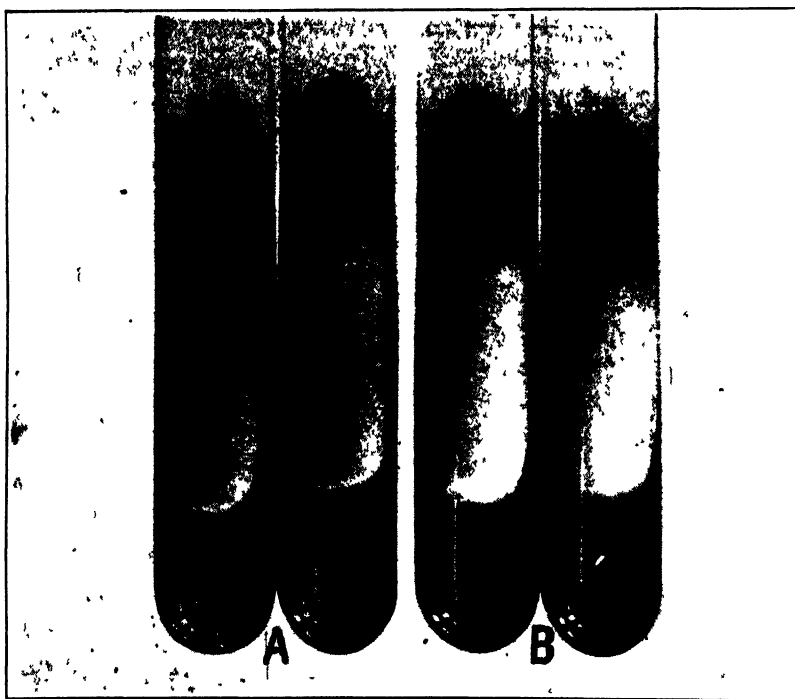


FIG. 4. A comparison of *Rhizopus tritici* (A) and *R. nodosus* (B) in culture on potato dextrose agar slants.

³ Formerly Associate in Crop Production, University of Illinois.

in the milk stage, were gathered, cured and tested for disease. The percentages of scutellum rot are given in table 3. The ears that were harvested in the milk stage in 1924 had only 6.9 per cent dead kernels, and the kernels from the same ears produced 42.5 per cent strong seedlings. With advancing maturity there were fewer dead kernels and more strong seedlings. In 1925 the highest percentage of dead kernels was 2.3, in the glaze stage. In both seasons the vigor of germination increased with maturity up to the dent stage, where it attained its maximum.

The percentage of scutellum rot was very high in the corn harvested in the milk and glaze stages. It was lower in the dent stage, and very much lower in the mature stage. While the maximum vigor had been attained in the dent stage, susceptibility to scutellum rot was still very high as compared with the more mature stage. Some important changes relative to resistance to the scutellum rot disease occurred during that time.

Similar data were obtained from another lot of yellow dent corn which was harvested at three different stages of maturity. There were 72 ears in each lot. The percentages of scutellum rot were as follows: glaze stage, 56.9 per cent; dent stage, 45.8 per cent; and mature stage, 32.4 per cent. Mature corn therefore is more resistant to this disease than immature corn.

Character of the Endosperm

In speaking of the character of the endosperm in this paper, only the outward appearances with respect to starchiness were considered. The separations were made into classes as illustrated by Trost (16). This same author has found starchy seed corn to be inferior to horny seed in its field performance. Starchy seed also showed the highest percentage of infection on the germinator, the causal organisms mentioned being *Fusarium* spp., *Diplodia zeae*, and *Penicillium* spp.

Holbert *et al* (8) have shown that starchy seed is more likely to be diseased than horny seed when tested on the germinator, and that, when nearly disease-free starchy seed was compared with nearly disease-free horny seed in field tests, greater reductions in yield occurred from the former seed when it was inoculated at planting time with *Gibberella saubinetii* or when it was planted on disease-infested soil.

It was shown by Koehler and Pettinger (10) that, in groups of corn differing in respect to endosperm composition, scutellum rot was most prevalent in the starchy groups. Other corn diseases found in seed corn on the germinator were not influenced by kernel composition in a similar manner.

The ears gathered at different stages of maturity, data on which are given in table 3, were classified with regard to the composition of the endosperm. The corn gathered in the milk stage was very starchy, most of it being of the A class according to Trost's classification. Of the ears har-

vested in the glaze stage, most were in the C class. The amount of scutellum rot infection was practically the same in the first two groups. In the dent stage most of the ears were in class E, and the amount of scutellum rot diminished about 20 per cent. In the mature and husking stages the ears were a little more horny and the percentage of scutellum rot was considerably lower. In general, disease resistance increased with an increase in horniness of the endosperm, but the two were not exactly parallel.

TABLE 3.—*The relation of kernel composition and percentage of scutellum rot in corn harvested at different stages of maturity and classified into six classes, "A" denoting very starchy endosperm, and "F" very horny endosperm*

Year	Stage of development	Number of ears in each class						Scutellum rot in per cent
		A	B	C	D	E	F	
1924	Milk	45	17	8				53.2
	Glaze	5	16	24	15	7	3	54.2
	Dent		2	6	9	34	14	43.5
	Mature			4	14	36	16	20.5
	Husking				14	40	16	19.4
1925	Glaze	4	20	25	31	9	1	21.3
	Dent		1	8	14	36	22	14.1
	Mature				12	39	28	8.4
	Husking				1	25	18	6.1

Data on another group of yellow dent corn ears show a close correlation between endosperm composition and scutellum rot (table 5). All of these ears were mature when harvested. There were variations in endosperm composition and, after the germination tests were made, the ears were divided into four classes with respect to starchiness, namely: C, D, E, and F. There was none that classified as A or B. Percentages of scutellum rot in the different classes were as follows: C, 47.4; D, 39.2; E, 34.0; and F, 29.1. The correlation between the two factors here is very close.

From data obtained and observations made during a number of years, it is evident that starchy seed is much more susceptible to scutellum rot than horny seed. Furthermore, as far as resistance or susceptibility is evident on the germinator, no other disease is similarly influenced by variations in starchiness of the kernels. In the soil, starchy seed also is more susceptible to disease, but whether here it is entirely a scutellum rot relationship is not known. Several soil fungi, however, were isolated from typical scutellum rot conditions in seedlings grown in soil (table 2).

Curing Conditions

The principal object of these experiments was to determine whether a greater degree of disease resistance could be obtained by drying the corn more rapidly than is ordinarily done. No experiments were conducted under poor curing conditions.

In one experiment a lot of ears were divided into four similar groups of 54 ears each. One lot was dried at room temperature ranging from 20° to 25° C. The other lots were dried in hot air currents maintained at temperatures of 39.5° to 45.0°, 42.0° to 47.5°, and 47.5° to 56.5° C. The drying process at these temperatures was extended over a period of 48 hours.

When tested for susceptibility to scutellum rot, the various groups were nearly the same. No benefit was derived through rapid drying.

TABLE 4.—*Effect of different drying conditions on the subsequent susceptibility of yellow dent corn to the scutellum rot disease*

Drying conditions	Percentage Moisture				Percentage of scutellum rot	
	Oct. 21	Oct. 28	Nov. 4	Jan. 24	Without inoculation	After inoculation with <i>R. nodosus</i>
In well ventilated room at about 22° C. (240 ears)	34.2	14.9	12.1	11.9	12.8	34.6
Under roof at outdoor temperature (240 ears)	34.0	25.1	18.5	14.8	8.3	34.3

In another experiment 480 ears were harvested in the mature stage on October 21, 1924, from green, apparently healthy stalks. Half of these were laid in wire hangers and hung in one of the farm buildings. The windows were kept open so as to allow plenty of ventilation. The other half of the ears was placed into similar hangers, but hung in a heated building. Some of the windows were also kept partly open during the first month to permit ventilation. Moisture tests were made of this corn at the time of harvest and at three later periods. The data are shown in table 4. The initial moisture content was 34.0 to 34.2 per cent. In the warm room this was reduced to 14.9 per cent during the first week. At the outdoor temperature three months were required to dry the corn to the same extent. When disease readings were made on the germinator in the ordinary way, the corn that was dried quickly showed the most scutellum rot. But when all the kernels were sprayed with a spore suspension of *Rhizopus*, the disease readings were practically identical. Evidently the corn dried indoors was more subject to contamination with *Rhizopus* spores; but when all of it was inoculated so that all had equal opportunities, it became evident that

these two methods of curing had no differential effect on the corn's resistance to scutellum rot.

Diffusions from Germinating Corn Kernels

Observations on the germinator (Fig. 1) and under pure culture conditions have shown that the molds grow much better on some corn kernels than on others. As *Rhizopus* belongs to a group of organisms known as weak parasites, it seemed likely that the organisms did not need to penetrate into the scutellar region before coming into contact with their food supply, but that during the germinating process food materials were diffused from these kernels which gave the fungi their initial start. Consequently some experiments were planned to determine whether (1) during the germinating process substances were diffused from the kernels which support growth of organisms that may cause scutellum rot; (2) whether there is any difference in this respect between seedlings resistant and susceptible to scutellum rot; and (3) if there is a difference, to determine whether it is due to the relative amount of total matter diffused or whether it is due rather to the specific character of these substances.

Petri dishes were prepared with sterile non-nutrient agar. Test dishes inoculated with *R. nodosus* developed no growth beyond the spore germination stage. Disease-free, surface-sterilized kernels of corn susceptible to scutellum rot were placed in one set of dishes, five kernels to a dish. Disease-free, resistant corn kernels were treated similarly and placed in another set of dishes. This corn was germinated at room temperature until the plumules were about two inches long. Then all the seedlings were removed from the dishes, and spores of *R. nodosus* were sown on the agar.

Considerable fungous growth occurred in the plates in which the susceptible seed had germinated (see Fig. 5), but very sparse growth occurred in those plates in which the resistant kernels had germinated. This showed that the substances passing out from the kernels during the germinating process supported *Rhizopus* growth well in one case, but very poorly in the other. This indicated two possibilities: either considerable food material passed out in the one case and very little in the other, or food materials passed out in both cases, but a toxic substance was also present in the agar upon which the resistant seed was germinated.

Corn kernels were germinated in flasks of distilled water. It was found that when resistant seed was used, the water became slightly turbid; but when susceptible seed was used, the water became considerably more turbid. When the kernels were partly submerged, they germinated very well; when entirely submerged, they did not germinate. It was found that the turbidity was greater when the kernels were allowed to germinate than when they did not germinate. Neither the kernels nor the water was sterilized. By

evaporating the solutions to dryness, it was also found that more solid matter had diffused from the germinated kernels than from those that had not germinated.

A group of seed ears was divided into four classes with respect to starchiness. (See table 5.) Class C was somewhat starchy, while Class F was very horny, as already explained. In each group, the 10 most susceptible and the 10 most resistant ears were selected. Representative kernels from these 10-ear groups, as well as from the groups as a whole, were taken for making extractions by the diffusion method. Twenty grams of corn kernels and 70 cc. distilled water were placed in 150 x 20 mm. petri dishes. They were made up in triplicate and were incubated for three days at 30° C. Good germination of the corn was secured. At the end of that time the liquids were filtered through coarse filter paper (Whatman No. 4) and tested for crystalloids with an Abbe refractometer, and for turbidity with a Kleinman nephelometer. A 0.2 per cent cornstarch solution was used as a standard colloidal solution against which the turbidity of the liquids was

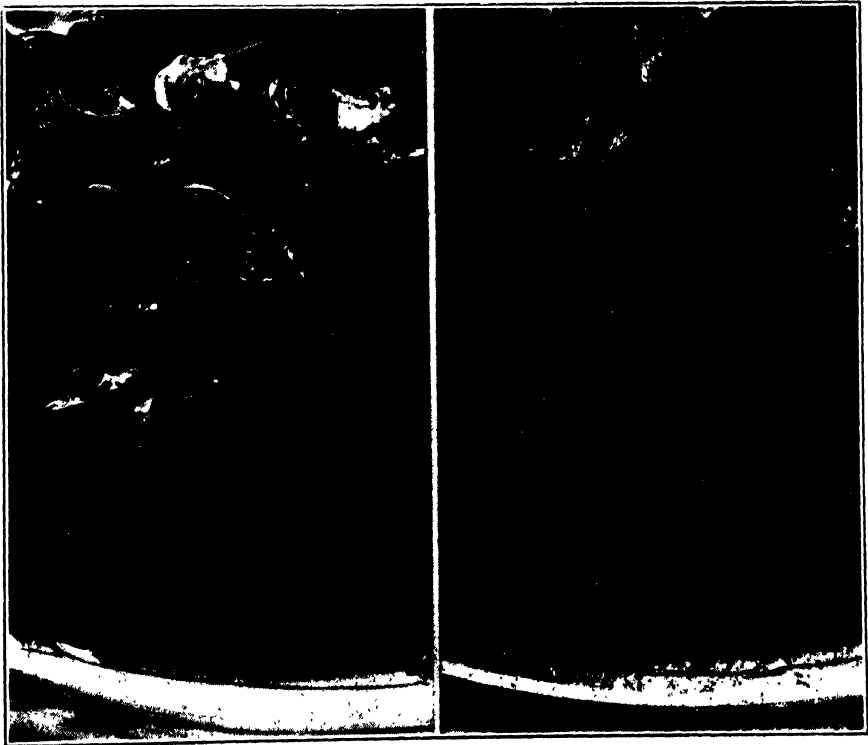


FIG. 5. *Rhizopus* growth on non-nutrient agar on which, at the left, resistant corn kernels had germinated and, at the right, susceptible kernels had germinated. The kernels were removed before inoculation with *Rhizopus*. The fungus grew on the nourishment diffused from the kernels. Enlarged.

checked. The solution was made by boiling the starch in distilled water for six hours under a reflux condenser, adding mercuric chloride as a preservative, allowing it to stand for a week, and decanting the upper liquid. The results of these readings as well as the percentage of scutellum rot in each group of corn as found on the germinator are given in table 5.

The refractometer showed no differences between the solutions from the various corn selections. There were only small amounts of solutes in the solution. A check reading made with pure water registered an index of 1.338 under the same temperature conditions.

The nephelometer readings proved very interesting. It must be borne in mind that the lower the scale reading the higher the turbidity of the solution. The lowest reading (highest turbidity of the solution in which the corn was germinated) was practically equivalent to a 1.0 per cent starch solution. It should be noted first that within each grade of starchiness the most turbid solutions came from the susceptible seed and the least turbid from the resistant seed. Furthermore, with decreasing soft starch in the endosperm, both disease susceptibility and turbidity of the liquid decreased.

Microscopic examination showed that the turbidity was due, at least in large measure, to bacterial flora. The experiment was repeated except that the corn kernels were first sterilized according to the method already described, and the diffusions were made under sterile conditions. No turbidity occurred in the solutions. Ears infected with *Fusarium moniliforme*, how-

TABLE 5.—*Refractometer and nephelometer readings of culture solutions and percentages of scutellum rot in four lots of corn, differing with respect to degree of starchiness*

Grade of starchiness	Ear selections	Number of ears	Refractometer index	Nephelometer readings in mm.		Scutellum rot in per cent
				I	II	
C	Most susceptible	10	1.3342	5.5	5.0	79.0
	Whole sample	47	1.3342	6.0	5.2	47.4
	Most resistant	10	1.3341	8.5	8.6	27.0
D	Most susceptible	10	1.3341	7.3	6.0	82.0
	Whole sample	97	1.3342	8.5	8.0	39.2
	Most resistant	10	1.3340	17.0	15.0	5.0
E	Most susceptible	10	1.3343	6.0	7.0	76.0
	Whole sample	257	1.3342	10.0	10.5	34.0
	Most resistant	10	1.3341	11.5	11.7	3.0
F	Most susceptible	10	1.3342	8.5	9.5	68.0
	Whole sample	85	1.3341	13.5	10.7	29.1
	Most resistant	10	1.3341	15.7	13.5	2.0

ever, had to be rejected because a 90-minute treatment with a 0.5 per cent Uspulun solution does not kill this fungus. These results seemed to demonstrate that the turbidity was due entirely to living organisms and not to materials diffused from the kernels.

Again, it became evident, just as in the previous experiment where kernels were germinated on non-nutrient agar, that in the case of the scutellum rot-susceptible kernels, something diffused which was favorable to the growth of organisms, in this case bacteria; whereas from resistant seed, either very little diffusion of materials suitable for bacterial growth occurred, or some chemical inhibitor was present.

An experiment was planned in which the total amount of solid non-volatile material diffused from the susceptible and resistant kernels could be compared. Eight ears each of five kinds of disease-free corn were selected, namely (1) Reid's Yellow Dent resistant to scutellum rot, horny endosperm; (2) Reid's Yellow Dent susceptible to scutellum rot, horny endosperm; (3) Reid's Yellow Dent, susceptible, but with starchy endosperm; (4) an inbred line, resistant, and (5) an inbred line susceptible to this disease. Some information on the history and field performance of these inbred strains has already been published (9, pp. 354-6). The two inbred strains compared here are very much alike in size, shape, and texture of kernels.

As in the previous work, 20 grams of corn were placed in 70 cc. distilled water in large petri dishes and allowed to germinate under sterile conditions. Three dishes were made up from each ear. After 3 days in the case

TABLE 6.—*Weights of extracted solid matter from five kinds of corn, the extractions having occurred while 60 grams of corn were germinated in 210 cc. distilled water under sterile conditions*

Ear No.	Dry weight in grams of extracted solid matter				
	Resistant Reid's Yellow Dent, horny endosperm	Susceptible Reid's Yellow Dent, horny endosperm	Susceptible Reid's Yellow Dent, starchy endosperm	Resistant Pure line No. A-1-1-2- R-1-1-9-1	Susceptible Pure line No. A-1-1-4- 1-8-1-5-1
1	0.192	0.150	0.453	0.791	0.163
2	0.257	0.177	0.648	0.822	0.190
3	0.322	0.203	0.364	0.817	0.231
4	0.307	0.195	0.429	0.762	0.196
5	0.230	0.245	0.520	0.812	0.210
6	0.214	0.235	0.378	0.848	0.244
7	0.196	0.210	0.407	0.743	0.132
8	0.202	0.185	0.485	0.794	0.171
Averages	0.240	0.200	0.461	0.799	0.192

of the Reid's Yellow Dent corn and 4 days in the case of the inbred corn, the solutions from all three dishes were transferred to a beaker and evaporated to a small volume at 85° C. This solution was then transferred to a tared weighing bottle and dried down to constant weight. The results are given in table 6. Not all of these could be handled at one time. Only two ears of each of the five kinds of corn were used at each operation.

It is evident that the total matter diffused from susceptible seed is not greater than that from resistant seed when both groups are similar in respect to horniness of the endosperm. The starchy seed did show a uniformly greater amount of solid material in the culture solution than did the horny seed. This may account in part for the fact that starchy seed usually is more susceptible than horny seed.

In the inbred strains the amount of solid matter in the culture solution from the susceptible line was even very much less than from the resistant line. This is the reverse of what would occur if resistance or susceptibility



FIG. 6. *Rhizopus* growth on corn extracts; left, from susceptible seed; right, resistant seed. The extracts were made by germinating the corn in sterile water. These solutions were added to sterile sand in test tubes and then inoculated with *Rhizopus*. Enlarged. Note the difference in growth.

were governed by the amount of material that diffuses from the germinating kernels. Therefore it is probably not the quantity of this material but its specific character that influences the expression of resistance or susceptibility to the disease.

In another experiment, sterile culture solutions from the resistant and susceptible inbred lines were placed on heat-sterilized sand in test tubes, 5 cc. liquid to 18 cc. dry sand. These tubes were then inoculated with *R. nodosus*. In the tubes with solutions from susceptible seed the fungus grew well; but in the other tubes, although the amount of solid matter diffused out was greater, there was very poor growth (Fig. 6). In tubes similarly prepared, but heated in the autoclave for 30 minutes at 15 pounds pressure before inoculating, there was good fungous growth in both sets, the fungus, however, still growing better in the tubes with solutions from susceptible seed. This experiment was repeated, except that resistant and susceptible open-pollinated seed was used. The results were the same.

Something happened during the autoclaving process that changed the extract from the resistant seed. Either something was removed by distillation or some physico-chemical change occurred that made the extract more suitable as a nutrient material for the fungus.

SUMMARY

Scutellum rot of corn is a disease that occurs during the seedling stage of the plant and may be caused by a number of different organisms. On the germinator, *Rhizopus* spp. are the most common causal agents. The initial attack is most common in the epithelial region of the scutellum, but often also occurs at other points.

Inoculations with 12 species of *Rhizopus* at temperatures of 16°, 22° and 30° C. showed that all were able to cause scutellum rot at these temperatures, but differences in virulence were noted.

Isolations from *Rhizopus*-infected kernels on the germinator showed that *R. tritici* and *R. nodosus* were the predominating species under the conditions studied. Infection with *R. nigricans* was not found.

Isolations from seedlings grown from susceptible seed in soil at 16° C. showed that species of *Mucor*, *Penicillium*, and *Fusarium* were the predominating organisms associated with scutellum rot. Only about 10 per cent of the diseased kernels were infected with *Rhizopus*.

Immature seed was found to be more susceptible to scutellum rot than mature seed. The former seed was also found to be much more starchy in endosperm texture than the latter. This was perhaps the principal factor concerned.

In mature, field selected, seed corn, the starchy ears were more susceptible to scutellum rot than those with horny endosperm.

Slow drying of corn at outdoor temperature versus rapid drying at various warmer temperatures did not influence the resistance or susceptibility to scutellum rot.

During the germination process, especially after the pericarp breaks, substances diffuse from the kernels into the surrounding water. This occurred with all kernels tested, resistant or susceptible. In the case of susceptible kernels the extract obtained by the diffusion process was a suitable medium for vigorous growth of *Rhizopus*. The extract from the resistant seed induced a very poor growth of *Rhizopus*.

The substances emanating from germinating susceptible kernels, therefore, seem to stimulate vigorous growth of certain organisms on the exterior of the kernel or in the surrounding soil. These would grow most actively in the direction of the greatest concentration of the food substances diffusing from the kernel at the place the pericarp has ruptured. Entry of the kernel is then effected, usually at the margins of the scutellum.

As no substances suitable for fungous growth diffuse from germinating resistant kernels, there is little fungous growth on the kernel and subsequently into the kernel.

When the substances extracted by the diffusion process from susceptible and resistant kernels were evaporated down to dryness, no quantitative difference was found that would account for differences in resistance or susceptibility. The differences in these extractions, therefore, must be qualitative rather than quantitative.

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EFFECT OF EARLY SPRAY AND DUST APPLICATIONS ON LATER INCIDENCE OF CUCUMBER WILT AND MOSAIC DISEASES

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INTRODUCTION

The bacterial wilt (*Bacillus tracheiphilus* E. F. S.) and mosaic of cucumber are two diseases of major importance that are said to be largely, if not entirely, disseminated by insects. Of the several carriers concerned, Doolittle and Walker (2) found the striped cucumber beetle (*Diabrotica vittata* Fab.) to be the most important in transmitting the virus of mosaic from wild host plants to the cultivated cucumber. Rand and Enlows (3) and Doolittle (1) report that this same insect is responsible for the overwintering and the primary spread of the wilt disease. Thus we have a single primary carrier for both diseases. It appears reasonable to suppose that elimination of the chief means by which these diseases are introduced into the cucumber fields would be an effective method of control. How effective, however, seems to be a moot question, since the method has apparently not been tried out to any extent under controlled field conditions.

The present investigation was begun with the object of determining: (a) the value of the best entomological recommendations for striped beetle control, when faithfully followed, in restricting the occurrence of wilt and mosaic; (b) the effect of the addition of a fungicide (bordeaux) to the insecticide as a means of increasing its disease control value.

EXPERIMENTAL PROCEDURE

The work was carried out during 1925 and 1926 in eastern Long Island, where both diseases are very prevalent and destructive. All spray applications were made by the writer, and the work has been thorough and timely. The first treatments were given immediately after the first beetles appeared and dust treatments were renewed after each rain. The spray treatments were renewed as the growth of the cucumber vines required.

In the 1925 experiment, 27 plats were laid out, each consisting of a single row 66 feet long, with the plants spaced a foot apart. Seed was sown on June 3. The special beetle control treatments were applied during the first 5 weeks of growth. After that time, the plants having reached the vining stage, regular applications of bordeaux spray were given at 5- to 10-day intervals. In 1925 during the initial 5-weeks' period the following treatments were compared.

Series A: Sprayed with bordeaux mixture (2-4-50) + 3 lbs. arsenate of lead and $\frac{1}{2}$ gallon miscible oil for spreader. Five applications were made in all: June 15, 19, 25, 30, and July 6.

Series B: Dusted with arsenate of lead and lime (1 to 15). Seven applications were made: June 16, 19, 22, 26, 29, 30 and July 6.

Series C: Given only sufficient protection to prevent loss of plants from the excessive feeding of the insects. One application of dust was necessary, and was given on June 22.

Treatment A was decided upon as the result of preliminary trials. Treatment B was recommended to the writer by the entomologist as the most satisfactory control for beetles known at the time.

In the 1926 experiment, certain modifications were introduced. First the size of the plats was greatly increased. In place of a single 66-foot row, six rows each of 36-foot length were used. This made a rectangular block 24 feet wide, with each block separated on sides and end by a 12-foot interval. The seed was sown in hills with 12 hills to a row and 3 plants to a hill. The date of sowing was June 17, and the special treatments covered the subsequent 6-weeks period. All plats after this were given regular and uniform applications of bordeaux mixture.

Series A received bordeaux mixture (2.5-5-50) + 3 lbs. arsenate of lead and $\frac{1}{2}$ gallon miscible oil for two applications and then 4-6-50-3-0.5 for two more. The dates of applications were July 7, 12, 19 and 23.

Series B received calcium arsenate-gypsum dust (1-15) for five applications: July 7, 12, 16, 19, and 23.

Series C received a single dusting, merely to keep the beetles from eating the young plants. This was applied on July 12.

The calcium arsenate-gypsum dust was substituted for the lead arsenate-lime combination on the recommendation of the entomologist. The 2-4-50 bordeaux was increased in strength to determine whether better disease control could not be secured. Thus Rand and Enlows (3) recommend a 4-5-50 bordeaux as being better than weaker solutions.

During the two seasons a number of other diseases and insects were encountered, but in no case were they serious factors. The most bothersome was a thrip infection in 1925 which threatened for a short time to become serious. A thorough treatment of nicotine dust applied in the middle of a warm day reduced this trouble very satisfactorily. In 1926 aphids were troublesome, and nicotine was used in one of the general sprays with fairly satisfactory results. The only disease in addition to wilt and mosaic that assumed any serious proportions was powdery mildew caused by *Erysiphe cichoracearum* D. C. This trouble was encountered late in the summer of 1926 and was only partly controlled by bordeaux spraying.

TABLE 1.—*Effect of early spray and dust treatments on the incidence of bacterial wilt of cucumber in 1925*

Plot no.	Variety	Treatments ^a	No. plants killed by wilt	
			July 6	July 17 ^b
SERIES A				
1	Kirby	Sprayed with bordeaux mixture (2-4-50) plus 3 lbs. lead arsenate plus $\frac{1}{2}$ gal. oil. Five applications.	0	2
2	do		0	2
3	do		0	0
4	Boston Pickle		1	0
5	do		2	0
6	do		0	0
7	Chicago Pickle		0	1
8	do		0	0
9	do		0	1
			Total	9
SERIES B				
1	Kirby	Dusted with arsenate of lead and lime (1-15). Seven applications.	0	0
2	do		0	2
3	do		1	3
4	Boston Pickle		2	0
5	do		0	2
6	do		0	3
7	Chicago Pickle		0	1
8	do		1	0
9	do		0	0
			Total	15
SERIES C				
1	Kirby	Checks. One application of dust to prevent excess feeding of insects.	1	14
2	do		6	7
3	do		6	9
4	Boston Pickle		3	6
5	do		5	4
6	do		3	4
7	Chicago Pickle		2	6
8	do		4	9
9	do		1	7
			Total	97

^a In each plot one 66-foot row of plants, one foot apart, was treated.

^b The disease continued active in Series C after this date, but further counts were not made.

TABLE 2.—*Effect of early spray and dust treatments on the incidence of cucumber mosaic in 1925*

Plot no.	Variety	Treatment ^a	No. pickles picked										
			July 27		July 30		Aug. 3		Aug. 7		Aug. 11		
			H ^b	M	H	M	H	M	H	M	H	M	
SERIES A													
1	Kirby	Sprayed with bordeaux mixture (2-4-50) plus 3 lbs. arsenate of lead plus ½ gal. oil. Five applications.	35	1	51	0	78	6	55	34	40	27	
2	do		29	0	23	2	42	8	44	32	38	25	
3	do		31	2	28	3	62	19	55	21	31	28	
4	Boston Pickle		22	3	35	2	30	9	25	54	18	32	
5	do		15	0	26	0	45	5	32	33	36	38	
6	do		11	0	22	0	59	9	41	20	23	27	
7	Chicago Pickle		20	1	29	2	32	10	29	36	29	26	
8	do		31	2	47	2	59	9	30	36	42	32	
9	do		20	1	26	1	44	14	35	28	19	21	
Totals			214	10	287	12	451	89	346	294	276	256	
Average percentage mosaic			4		4		16		46		48		
SERIES B													
1	Kirby	Dusted with arsenate of lead and lime (1-15). Seven applications.	8	4	25	0	37	1	29	22	38	34	
2	do		17	2	14	1	31	5	28	11	36	22	
3	do		21	0	55	2	49	12	50	41	36	26	
4	Boston Pickle		16	9	20	7	24	21	12	28	28	45	
5	do		16	0	14	0	40	14	15	20	27	31	
6	do		14	0	22	5	44	13	19	36	28	22	
7	Chicago Pickle		15	7	26	3	30	10	26	29	28	22	
8	do		24	1	33	1	35	8	28	23	17	26	
9	do		21	0	27	1	34	8	30	28	28	26	
Totals			152	23	236	20	324	92	237	248	266	254	
Average percentage mosaic			13		8		22		51		49		
SERIES C													
1	Kirby	Checks. One application of dust to prevent excess feeding of insects.	21	1	14	7	15	10	12	33	10	12	
2	do		4	0	10	3	9	6	11	13	21	13	
3	do		10	1	4	1	11	2	4	15	10	20	
4	Boston Pickle		23	0	6	1	16	14	7	31	6	25	
5	do		0	1	2	1	7	3	11	5	6	3	
6	do		4	0	6	1	7	7	7	4	15	8	
7	Chicago Pickle		14	9	19	2	14	12	7	28	13	24	
8	do		5	2	12	0	11	9	20	16	21	14	
9	do		13	0	12	0	8	9	15	13	13	9	
Totals			94	5	85	16	98	72	94	158	115	128	
Average percentage mosaic			5		16		42		62		53		

^a In each plot one 66-foot row of plants one foot apart was treated.^b H denotes healthy pickles; M denotes mosaic pickles.

TABLE 3.—*Effect of early spray and dust treatments on the incidence of bacterial wilt of cucumber in 1926*

Plot no.	Variety	Treatments ^a	No. plants killed by wilt	
			July 29	Aug. 12
SERIES A				
1	Kirby	Sprayed with bordeaux mix-	0	3
2	do	ture (2.5-5-50) plus 3 lbs.	0	0
3	Boston Pickle	lead arsenate plus ½ gal.	0	0
4	do	oil. Two applications.	0	1
		Bordeaux mixture (4-6-50)		
		plus same amounts of lead		
		arsenate and oil. Two ap-		
		plications.		
			Total	
SERIES B				
1	Kirby	Dusted with calcium arsenate	0	1
2	do	and gypsum (1-15). Five	0	1
3	Boston Pickle	applications.	0	4
4	do		0	4
			Total 10	
SERIES C				
1	Kirby	Check. One application of	2	21
2	Boston Pickle	dust to prevent excess feed-	5	24
		ing of insects.		
			Total 52	
Checks doubled for comparison			104	

^a Each plot consisted of six rows 36 feet long. The plants were grown in hills 3 feet apart with 3 plants to the hill.

DISCUSSION OF RESULTS

In considering the four tables it should be pointed out first that wilt was very prevalent in 1925 and 1926 but especially so in 1925. Mosaic was very severe in 1925 but much less so in 1926. Consequently for the latter year totals only are given of the mosaic pickle counts (table 4). Infections were too few to make the data for individual pickings significant, as was possible in 1925.

Wilt Control.—The effective control of bacterial wilt both by spray (Series A) and dust (Series B) was striking each year, although the spray gave slightly better results. Since the difference, however, is not large when contrasted with the amount of disease in the check plats (Series C), it may be said that spray and dust were about equally satisfactory. It

TABLE 4.—*Effect of early spray and dust treatments on the incidence of cucumber mosaic in 1926*

Plot no.	Variety	Treatment ^a	No. healthy pickles picked												Total no. mosaic pickles
			August						September						
			12	16	20	27	3	7	10	13	17				
SERIES A															
1	Kirby	Sprayed with bordeaux mixture (2.5-5-50) plus 3 lbs. lead arsenate plus ½ gal. oil. Two applications. Bordeaux mixture (4-6-50) plus same amounts of lead arsenate and oil. Two applications.	85	135	101	184	225	63	167	116				4	
2	do		115	178	116	340	266	117	168	176				17	
3	Boston Pickle		51	140	53	137	139	115	213	187	78			1	
4	do		59	151	44	147	135	118	139	173	87			7	
Totals			310	604	314	808	793	413	687	652	165			29	
SERIES B															
1	Kirby	Dusted with calcium arsenate and gypsum (1-15). Five applications.	199	209	173	360	289	97	187	130				32	
2	do		164	201	110	336	259	136	150	153				46	
3	Boston Pickle		55	118	38	142	94	112	106	150	94			28	
4	do		99	144	44	160	120	106	124	159	60			6	
Totals			517	672	365	998	762	451	567	592	154			112	
SERIES C															
1	Kirby	Check. One application of dust to prevent excess feeding of insects.	73	115	55	235	181	97	127	113				61	
2	Boston Pickle		48	107	39	122	65	69	98	112	56			17	
Totals			121	222	94	357	246	166	225	225	56		78		
Checks doubled for comparison			242	444	199	714	492	332	450	450	112		156		

^a Each plot consisted of six rows 36 feet long. The plants were grown in hills 3 feet apart with 3 plants to the hill.

appears, in sections where wilt is a problem, that it is essential in controlling the striped beetle to consider more than the immediate damage due to its feeding. In these experiments the check plats (Series C) were given sufficient protection to prevent loss of plants through the feeding of beetles, but this degree of protection failed as a wilt preventive.

The early gathering and destruction of diseased plants is usually recommended as an important control measure for wilt. All wilt-infected plants found on the first inspection were removed but at the time of the second inspection the plants were too large and intertwined for this to be done. The removal of diseased plants has not appeared to have any noticeable effect upon the subsequent development of the disease, and the data from Series C indicate that it is of little value as a control measure.

Rand and Enlows (3) summarize their chief recommendations for field control of the wilt disease as follows:

“Where the disease is likely to be severe, spraying with strong bordeaux mixture and lead arsenate powder (4-5-50-2) is recommended. Treatments should begin as soon as the cucumber plants develop their first true leaves and should continue at intervals of about a week until the cucumber beetles practically disappear from the field. In localities where downy mildew is also prevalent the treatments should also be continued later as a partial insurance against this disease.

“The pulling of wilted vines during the first part of the season, or as long as it can be done without mechanically injuring the healthy plants, will greatly assist in controlling bacterial wilt if consistently done in all neighboring fields. The diseased vines should be buried or otherwise removed from access by the beetles.”

In the experiments here reported, the use of a bordeaux mixture stronger than 2-4-50 on young plants caused injury and reduced the yield. The wilt control secured with this weaker mixture was almost perfect, although in one year experimental plats were adjacent to a field alive with beetles and severely infested with wilt. The conclusion is that, if by careful spraying or dusting the striped beetles are effectively controlled, there will be very few wilt-diseased plants needing removal; if, on the other hand, due to lack of attention, the beetles are permitted to feed freely and spread the disease, no subsequent attention to the removal of diseased plants will prevent serious loss from the disease. Either spraying or dusting will satisfactorily control wilt if the work is thoroughly done.

Mosaic Control.—The results of 1925 indicated that the early sprays (Series A) temporarily checked the development of the mosaic disease. Thus for the first three pickings the sprayed plants average 4, 4 and 16 per cent mosaic; the dusted plants 13, 8 and 22 per cent mosaic; the check plants 5, 16 and 42 per cent mosaic. Later, however, the disease became general and these differences disappeared. The results of 1926 paralleled those of 1925. This year less than one-fifth as many mosaic pickles were gathered

from sprayed plants as from dusted or check plants. Taking the matter as a whole, it appears that the early sprays (Series A) were partly effective in preventing mosaic infection and the early dust treatments (Series B) had but little influence. However, the results of 1925 indicate that the degree of control which has been secured in these experiments is not sufficient to give any real protection against the mosaic disease since the disease spreads rapidly and sufficient infection occurs in spite of the early treatments to cause a maximum loss.

Effect of Spray or Dust Treatments on Growth and Yield.—In 1925 the bordeaux-sprayed plats out-yielded the dusted plats. The difference was most prominent in the early pickings. Thus the first picking from the nine dusted plats yielded about one-fourth less than the first picking from nine sprayed plats. This difference in yield was not correlated with any apparent differences in size of vines. The lime-arsenate of lead dust is, of course, liable to cause stunting if applied too heavily, and care was taken to apply it lightly and uniformly. Nevertheless the dusted plants did not have as good color as those which had been sprayed, they did not yield so well, and they began to die sooner in the autumn.

In 1926 a stronger bordeaux mixture was used and gypsum-calcium arsenate was used as a dust. The stronger bordeaux proved toxic and stunted the vines. The stunting was not extremely pronounced but could be easily detected. The dusted plats distinctly out-yielded the sprayed plats this year in the early pickings but even with this handicap the sprayed plats out-yielded the dusted in the last two pickings. The color of foliage in the dusted plats was as good as the color of the plants on the sprayed plats during the first two-thirds of the season, but this year again the dusted vines began to die first. This tendency of the dusted vines to die first in the fall is interesting in view of the fact that after the early treatments for beetle control all plats were given uniform bordeaux spray treatment for the remainder of the season. In 1926 it appeared that the early death of plants on the dusted plat was associated with greater susceptibility to powdery mildew.

SUMMARY

The chief primary agency for dissemination of the bacterial wilt and mosaic disease of cucurbits is the striped beetle, and the control of this insect requires applications of dust or spray during the first six weeks after planting.

Bacterial wilt may be effectively controlled by spraying with bordeaux mixture and lead arsenate or by dusting with lead arsenate-lime dust or calcium arsenate-gypsum dust. A thorough program of spraying or dusting to control beetles is a practical method of preventing loss from bacterial wilt.

The bordeaux and lead arsenate spray reduced the amount of mosaic distinctly; the dust treatments reduced the mosaic slightly; but none controlled mosaic satisfactorily.

The 2-4-50 bordeaux spray proved distinctly better than a stronger mixture, since the latter caused stunting and reduced yield. The calcium arsenate-gypsum dust (1-15) was a very satisfactory mixture.

There was a distinct tendency of plants given early application of bordeaux to remain green longer in the fall than plants given the dust treatments.

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THE EFFECT OF DIFFERENT HOSTS UPON THE SPORANGIA OF SOME PHYTOPHTHORAS¹

LEON H. LEONIAN

The genus *Phytophthora* is composed of some highly plastic fungi. Many morphological as well as physiological characteristics of these organisms are governed to a great extent by the environmental conditions. This fact has been sufficiently demonstrated by laboratory experiments. Environment, either in the laboratory or in nature, is nothing more than a combination of chemical and physical factors. Since a host plant also exerts such chemical and physical influences, it is to be concluded that, if an organism can be modified by laboratory conditions, there is no reason why similar modifications should not follow in nature, where changes are more abrupt and food factors more complex. With this idea in view the writer inoculated a number of fleshy fruits and vegetables with some eighty different cultures of *Phytophthora*. Green bananas, oranges, lemons, potatoes, tomatoes, peppers and egg-plants were successfully inoculated. About 90 per cent of the organisms were able to infect all of these hosts through wounds and to produce a rapid deterioration of the tissues. This omnivorous habit shows how futile it is to use host relationships as limiting factors in the specific differentiation of Phytophthoras. The symptoms in the majority of cases were uniformly alike. Sherbakoff (4) observed a uniform similarity of symptoms when he inoculated various hosts with *Phytophthora omnivora* (*P. terrestris*).

In spite of the ready infection of all hosts, many of the organisms produced sporangia in great abundance only on tomatoes, egg-plants and peppers. Whenever these reproductive bodies occurred, however, there were striking differences in their morphological features, their size and shape, the nature of their papillae, etc.

Fig. 1 shows the sporangia of *P. phaseoli* as found on the pods of lima-bean and on bell-peppers. The pepper was inoculated directly from the bean. These and subsequent drawings were made with camera lucida, and no attempt was made to select any unusual type; the proportions, therefore, are relatively correct.

Fig. 2 represents the sporangia of *P. capsici* as they were produced upon the following substrata: potassium nitrate solution, egg-plant, bell-pepper, and tomato. To secure fruiting under controlled conditions, a well-nour-

¹ Published with the approval of the Director of the West Virginia Experiment Station as Scientific Paper No. 35.

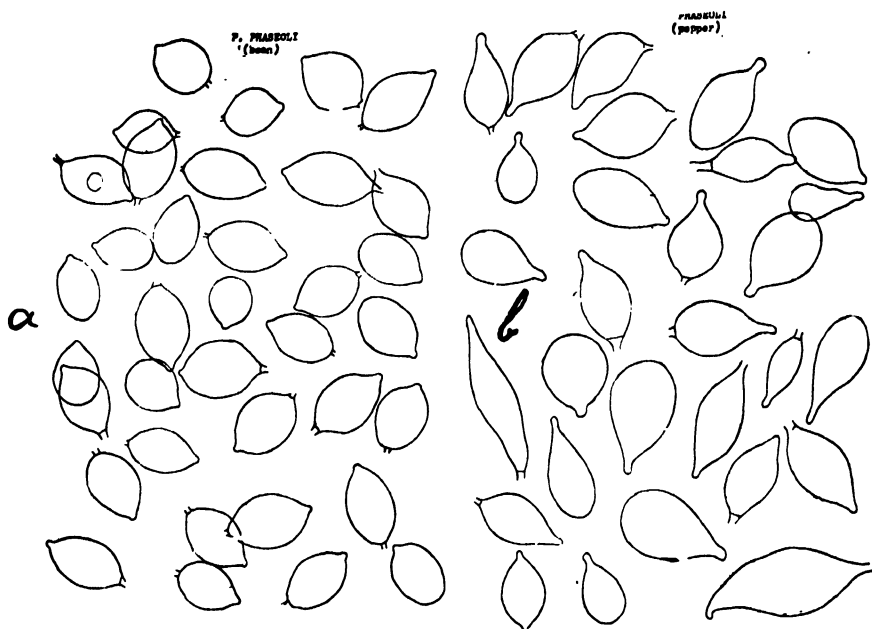


FIG. 1. Sporangia of *Phytophthora phaseoli*. a, on lima bean; b, on bell-pepper.

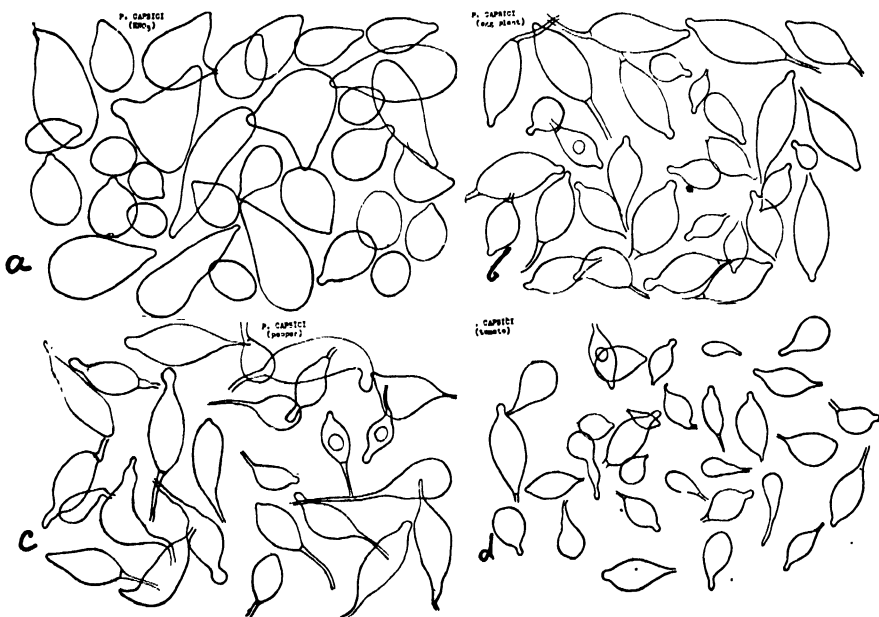


FIG. 2. Sporangia of *Phytophthora capsici*. a, on potassium nitrate solution; b, on eggplant; c, on bell-pepper; d, on tomato.

ished mycelium was washed in sterile distilled water and transferred to 2 cc. of M/100 potassium nitrate solution. After being kept at 25° C. for three days, the culture was examined for the reproductive bodies and the drawings were made. In case of host plants, sporangia were secured two days after the initial appearance of these bodies. This practice insured the exclusion of immature sporangia to a great extent.

Figs. 3, 4 and 5 represent the sporangia of three strains of *P. omnivora*

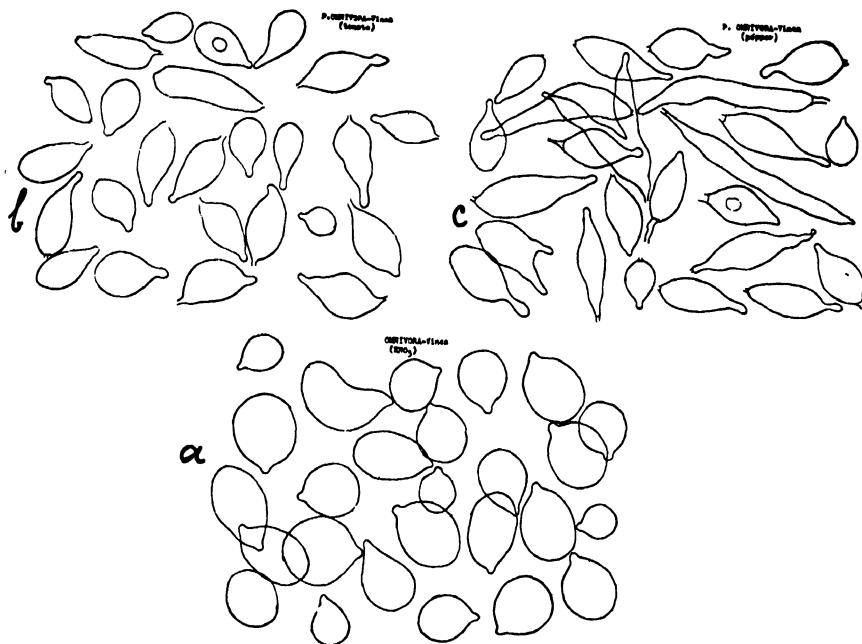


FIG. 3. Sporangia of *Phytophthora omnivora* (Vinea strain). b, on tomato; c, on bell-pepper; a, in potassium nitrate solution.

which were originally isolated from *Vinca*, tobacco and tomato respectively. This species is the most common and the most widespread representative of the genus. It is perhaps for this reason that a number of strains of this fungus were described as separate species by different workers, who felt themselves bound by the time-honored morphological standards. A glance at the various figures is sufficient to substantiate this statement.

Not only the different species of a given host plant but even two varieties of the same species exert entirely different influences upon the sporangia. Fig. 5 shows the tomato strain of *P. omnivora* "performing" on different hosts. On the mild bell-pepper the sporangia are very much elongated, and the papillae in most cases are drawn out thread-like. What a contrast between these and the reproductive bodies formed on the "hot" pepper! Fig. 6 shows the sporangia of *P. mexicana*. The differences are obvious.

The sporangia are very small and spherical on the tomato, very large and very elongated on the "hot" pepper; and what substantial papillae on the reproductive bodies formed on bell-pepper!

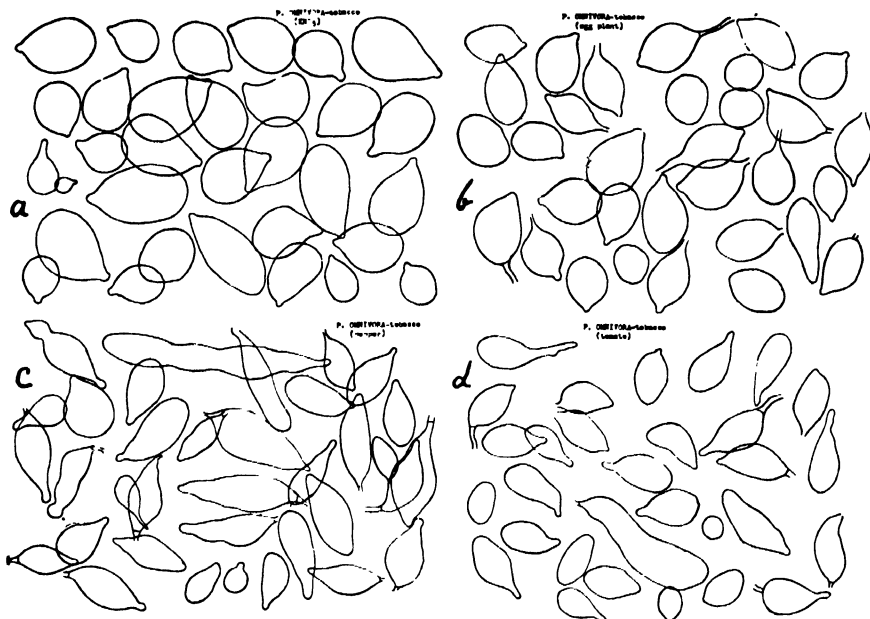


FIG. 4. Sporangia of *Phytophthora omnivora* (tobacco strain). a, in potassium nitrate solution; b, on egg-plant; c, on bell-pepper; d, on tomato.

It was observed that not only the different varieties of the same host but even different strains of the same variety were able to induce morphological modifications. This, however, is believed to be caused by the fluctuations in the environmental conditions rather than by the differences in the chemical composition of the host strains. Too much moisture, for example, checks formation of sporangia; a fairly abundant supply causes the production of larger sporangia; while a restricted supply of air moisture is conducive to much smaller reproductive bodies. The type of different saprophytes following the primary infection, and the nature of their by-products are probably important limiting factors in the morphological changes of the sporangia.

It may be deduced from the foregoing data that, so far as these organisms are concerned, no species can be described directly from the host; consequently type specimens and type species based on these specimens become obsolete. Nor can the investigator depend upon the so-called natural conditions which for such a long time have been considered by the taxonomist as the only safe and sane conditions. In the laboratory one should

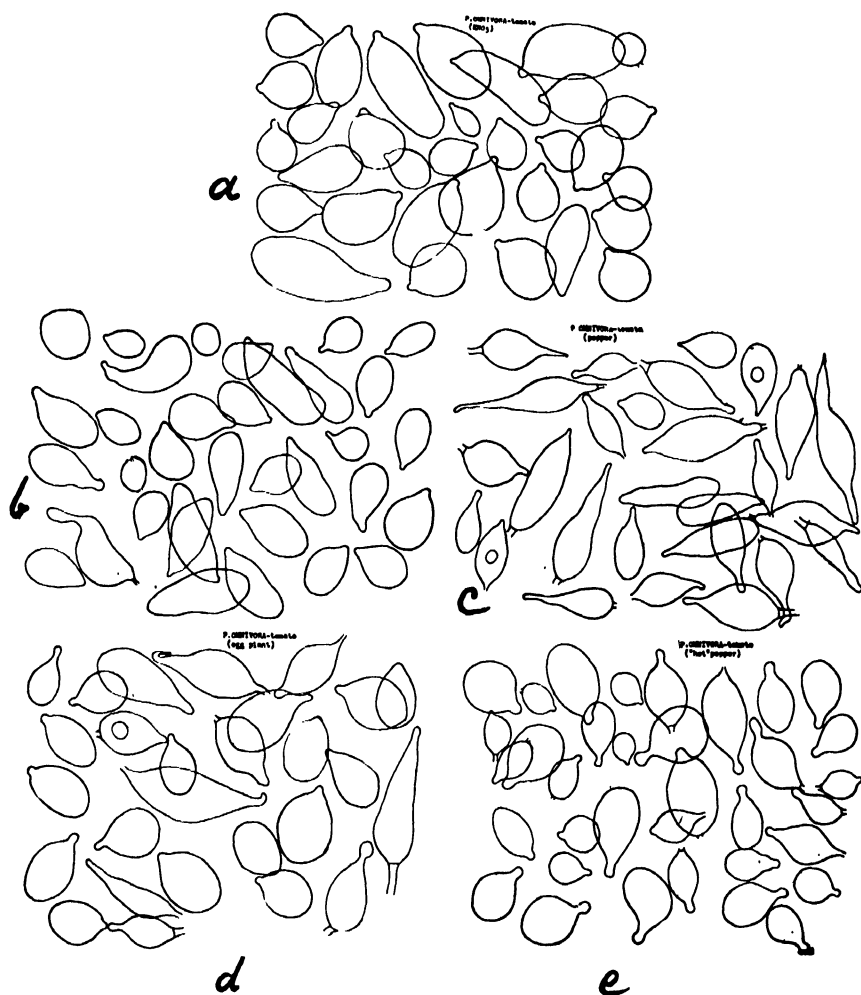


FIG. 5. Sporangia of *Phytophthora omnivora* (tomato strain). a, in potassium nitrate solution; b, on tomato; c, on bell-pepper; d, on egg-plant; e, on "hot" pepper.

be able to control conditions much better. It is true that no chemical compound is absolutely pure, that no temperature is always invariable, and no man-made technique infallible; but, on the other hand, temperature and moisture changes in nature are extremely variable, the competing micro-organisms and their by-products are too complex and not only two different hosts but even two different parts of the same host are much more unlike than any two chemical compounds which may be used in the laboratory. It is true that, after the fungus begins to work upon a medium of known quality and quantity, an unknown substance may result, but the same thing applies to the natural conditions to a greater extent.

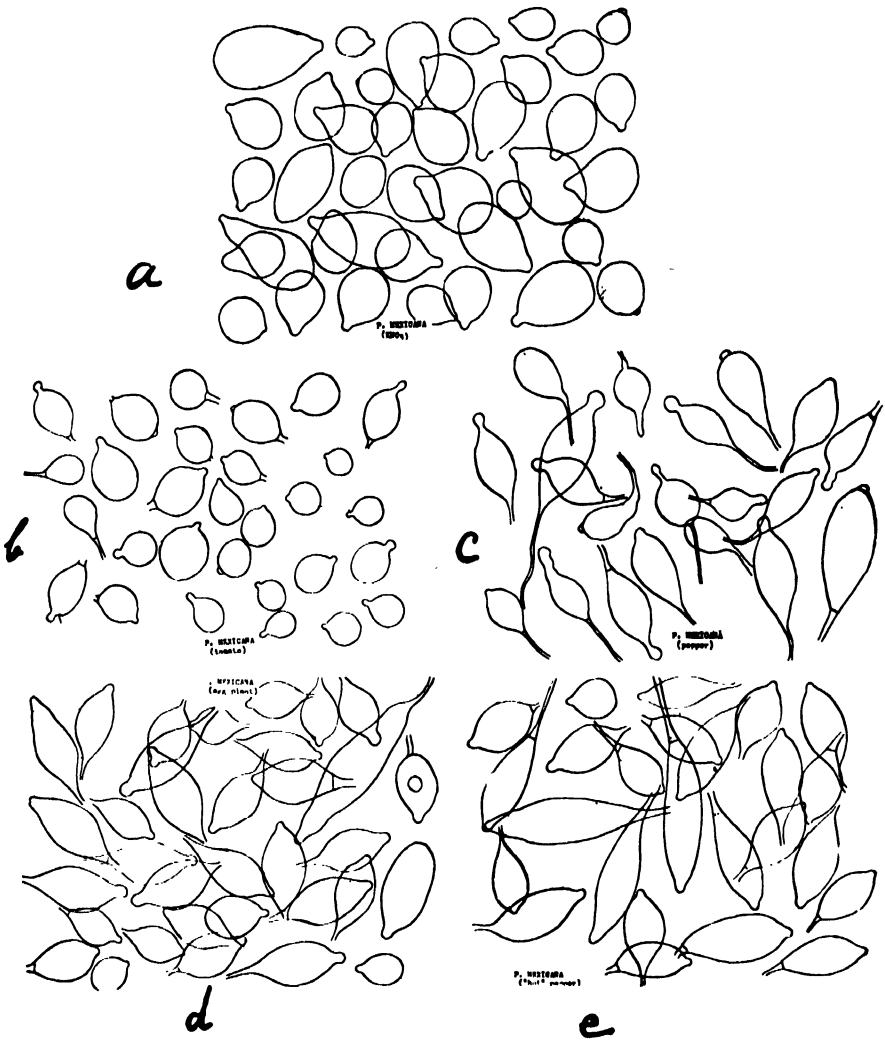


FIG. 6. Sporangia of *Phytophthora mexicana*. a, in potassium nitrate solution; b, on tomato; c, on bell-pepper; d, on egg-plant; e, on "hot" pepper.

If the final identification of a given *Phytophthora* is to be made in the laboratory, it is obvious that such complex media as oatmeal, cornmeal, prune juice and potato agars, which have been such great favorites of the plant pathologist, cannot be used. Nor can we consider the haphazard combination of chemicals known as Richard's solution and so commonly used whenever there is need of a synthetic solution. A simple solution of known quality and quantity should be preferred in all cases, and the conditions should be simplified still further by transferring a well-nourished mycelium to such a solution as M/100 potassium nitrate, which is known to stimulate

formation of sporangia. Such controlled conditions are infinitely more valuable than the haphazard methods which have been followed by many workers. This statement is based on some ten thousand measurements of the sporangia of 85 different *Phytophthoras*. However, no matter how carefully conditions are controlled and measurements made, a wide fluctuating margin should always be allowed because of the naturally plastic habit of these organisms. Measurements, therefore, together with other morphological data, can rarely possess sufficient merit to justify their use in the primary separation of species. Their cumulative value, however, can not be disregarded.

The writer wishes to make no generalizations concerning the effect of different hosts upon the morphology of fungi. Undoubtedly there are a large number of organisms which are not so plastic and can not be modified materially by a change of hosts. However, there are sufficient instances on record demonstrating that many other fungi can be made to alter their morphological features. Perhaps the most notable work along this line is that of Welles (7) on the genus *Cercospora*. Through many extensive experiments he found that the size and septations of conidiophores and conidia were modified by the environmental changes and by the effect of different hosts to such an extent that one species would readily pass for an entirely different species. Welles concludes that, so far as the genus *Cercospora* is concerned, morphological characteristics have no value. Taubenhause (6) showed that, when sweet potato was inoculated with *Diplodia gossypii*, the fungus assumed the characteristics of the supposed genera of *Lasiodiplodia*, *Chaetodiplodia*, *Botryodiplodia* and *Diplodiella*. The same was found to be true when sweet potato was inoculated with *Lasiodiplodia*. A mere change of host plants, therefore, produced or abolished generic differences. The writer (1) demonstrated by controlled laboratory experiments that in the case of some fungi the presence or absence of stromata as well as pycnidial walls was controlled by food factors, thus altering the relationships of not only different genera but even the position of Sphaeropsidales and Melanconiales. There are examples in rust fungi where morphological characteristics are by no means constant or reliable. Long (3) observed that the influence of host plants upon the morphological characters of *Puccinia ellisiana* and *P. andropogonis* was very remarkable. *P. ellisiana* infects both *Viola* and *Pentstemon*, but when it passes through the latter host its characteristics entirely change and it becomes like *P. andropogonis*. When species of *Viola* were inoculated with *P. andropogonis*, the ensuing fungus became, so far as could be determined, *P. ellisiana*. Stakman and Levine (5) and Levine (2) state that congenial hosts do not affect different biological forms of *P. graminis*, whereas uncon-

genial hosts tend to decrease the size of uredinia and spores. Other adverse environmental conditions were also found to have similar effects.

The foregoing data clearly indicate that morphological characteristics, at least in many fungi, should not form the exclusive basis for the separation of species.

SUMMARY

1. A number of fleshy fruits and vegetables were inoculated with 85 different cultures of *Phytophthora*.

2. Nearly all of these organisms brought about a ready infection.

3. While the symptoms in all cases were much alike, there were remarkable modifications in the morphology of the sporangia due to the influence of different hosts.

4. It is concluded that identification of *Phytophthoras* directly from the host is misleading, and that controlled cultural work in the laboratory should always be used as a more trustworthy method of identification.

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FURTHER GERMINATION TESTS WITH TELIOSPORES OF RUSTS

W. E. MANEVAL

In an earlier paper (5) the writer reported the results of germination tests with the teliospores of ten species of rusts. It was pointed out that, although the teliospores of these rusts generally require a more or less definite resting period before germinating, a few teliospores may germinate in December or earlier, especially after prolonged floating on water or after alternate wetting and drying. During the past four years numerous additional tests with teliospores of six of these species of rusts (*Puccinia asparagi*, *P. helianthi*, *P. menthae*, *P. ruelliae*, *P. sorghi* and *P. windsoriae*) have given similar results, and germination of four species (*P. asparagi*, *P. menthae*, *P. sorghi* and *P. windsoriae*) has been obtained one to two months earlier than previously reported. The other four species have not been tested again.

Recently Mains (3) has reported that he obtained slight to abundant germination of teliospores of *Puccinia antirrhini* Diet. and Holw. as early as December or January. These results agree with those previously reported by Hockey (1), who obtained 14 to 22 per cent germination of the same rust, after exposure to cold or freezing, in January, 1921. Mains and Jackson (4) also succeeded in germinating teliospores of *Puccinia dispersa* Erikss. and Henn. from August to the following April and of *P. triticea* Erikss. from December to the next spring. Hoerner (2), although he used both fresh and overwintered teliospores of *P. coronata*, failed to obtain germination.

In addition to the ten species of rusts reported on in a previous paper (5), the writer has made germination tests of teliospores of the following rusts:

- Melampsora medusae* Thuem. on *Populus deltoides* Marsh.
- Uromyces appendiculatus* (Pers.) Link on *Phaseolus vulgaris* L.
- U. aristidae* Ell. and Ev. on *Aristida oligantha* Michx.
- U. caryophyllinus* (Schränk.) Wint. on *Dianthus caryophyllinus* L.
- U. hedysari-paniculati* (Schw.) Farl. on *Desmodium* sp.
- U. howei* Pk. on *Asclepias syriaca* L.
- U. lespedezae-procumbentis* (Schw.) Lagh. on *Lespedeza* sp.
- U. spermacoces* (Schw.) Thuem. on *Diodia teres* Walt.
- Puccinia emiliae* P. Henn. on *Calendula officinalis* L.
- P. xanthii* Schw. on *Xanthium* sp.

All collections of these rusts were made at or near Columbia, Missouri. The teliospores of the last two species require no rest period before germinating. All excepting *P. emiliae*, *P. xanthii* and *U. howei* are eu-types. Three of the eu-types, *Melampsora medusae*, *U. aristidae*, *U. caryophyllinus*, are heteroecious and the others autoecious.

The germination tests were all in duplicate and, unless otherwise stated, were made by floating the spores on distilled water and incubating at room temperature in alternate light and darkness (day and night). In the case of *Melampsora* and all species of *Uromyces* excepting *U. appendiculatus* and *U. caryophyllinus*, the number of germination tests has been small; however, teliospores of four species, *U. aristidae*, *U. hedysari-paniculati*, *U. howei* and *U. lespedezae-procumbentis*, germinated in two to six weeks.

TABLE 1.—Germination of teliospores of eight different species of rusts

Rust species	Date of collection	Date tested	Incubation period in days	Germination ^a
<i>Melampsora medusae</i>	Jan. 15, 1918	Jan. 28, 1918	2-3	++++ ^b
<i>Uromyces appendiculatus</i>	Sept. 14, 1922	Sept. 16, 1922	28	+ ^c
	do	do	60	++++
	Nov. 11, 1922	Nov. 11, 1922	2	+
	Oct. 12, 1926	Oct. 30, 1926	50	++++
<i>U. hedysari-paniculati</i>	Sept. 2, 1923	Sept. 3, 1923	40	+
	do	do	86	+++
<i>U. howei</i>	Oct. 7, 1923	Oct. 11, 1923	79	+
<i>U. lespedezae-procumbentis</i>	Oct. 2, 1923	Nov. 14, 1923	17	5 per cent
<i>U. spermacoces</i>	Dec. 14, 1924	Dec. 15, 1924	15	+
	do	Apr. 14, 1925	8	+ ^d
	do	do	8	5-10 per cent ^e
<i>U. aristidae</i>	Oct. 13, 1925	Oct. 15, 1925	12	+
<i>U. caryophyllinus</i>	Nov. 1, 1925	Nov. 3, 1925	6	+
	Apr. 7, 1925	Apr. 7, 1925	15	+
	do	do	15	30 ± per cent ^e

^a 0 = no germination; + = less than 1 per cent; ++ = 1-5 per cent; +++ = 10 ± per cent; ++++ = 30-90 per cent.

^b Spores alternately wet and dry from Jan. 15 to Jan. 28.

^c Spores kept during the winter as herbarium material at room temperature or lower germinated well in 1-2 weeks the following April.

^d Spores kept as herbarium material at room temperature or lower from Dec. 14, 1924, to April 14, 1925.

^e In 0.5 per cent KH_2PO_4 .

Teliospores of *U. spermacoces* and of *Melampsora medusae* were collected in December and January respectively. Those of the former germinated in two to four days and of the latter, after alternate wetting and drying for two to three weeks, in two weeks. A few of the spores of *U. appendiculatus* collected in September, October or November in five different years (1922–1926) always germinated in one to four weeks. In table 1 are given the dates of collection and earliest germination, the incubation period, and certain other data regarding germination of teliospores of these eight rusts.

EFFECT OF LIGHT AND DARKNESS

At various times cultures of teliospores of *Puccinia emiliae* (Dec. 1, 1925; Dec. 21, 1925; Mar. 2, 1926) and of *P. xanthii* (Dec. 23, 1925; Mar. 2, 1926) were incubated in alternate light and darkness (day and night) and in continuous darkness. In all such tests a very high percentage of

TABLE 2.—*Effect of light and darkness on germination of teliospores of Uromyces caryophyllinus*

Series I

Date tested	period in alternate light and darkness	Germination ^a	Period in darkness	Germination ^a
Dec. 1, 1925	Dec. 1 to Dec. 7	tr	Dec. 7 to Dec. 9	++++
do	Dec. 1 to Dec. 9	+	Dec. 9 to Dec. 11	++++
do	Dec. 1 to Dec. 11	0	Dec. 11 to Dec. 13	+++
Dec. 9, 1925	Dec. 9 to Dec. 10	tr	Dec. 10 to Dec. 13	++++
do	Dec. 9 to Dec. 11	tr	Dec. 11 to Dec. 15	++++
do	Dec. 9 to Dec. 12	tr	Dec. 12 to Dec. 15	++++
do	Dec. 9 to Dec. 14	tr	Dec. 14 to Dec. 16	++++
do	Dec. 9 to Dec. 18	tr	Dec. 18 to Dec. 20	++++
Dec. 21, 1925	Dec. 21 to Dec. 23	0	Dec. 23 to Dec. 26	++++
Nov. 28, 1925	Nov. 28 to Dec. 7	+	Dec. 7 to Dec. 9	++++
Oct. 30, 1926	Oct. 30 to Nov. 5	0	Nov. 5 to Nov. 8	100 per cent

Series II

Date tested	Period in darkness	Germination ^a	Period in alternate light and darkness	Germination ^a
Dec. 1, 1925	Dec. 1 to Dec. 7	0	Dec. 7 to Dec. 9	+
Dec. 21, 1925	Dec. 21 to Dec. 23	0	Dec. 23 to Dec. 26	2 ± per cent
Oct. 30, 1926	Oct. 30 to Nov. 5	20 per cent		
Dec. 9, 1925	Dec. 9 to Dec. 11	0	Dec. 11 to Dec. 16	tr ^b
do	Dec. 9 to Dec. 13	0	Dec. 13 to Dec. 16	tr ^b
do	Dec. 9 to Dec. 16	0	Dec. 16 to Dec. 18	0 ^b

^a 0 = no germination; tr = trace; + = less than 1 per cent; ++ = 1–5 per cent; +++ = 10 ± per cent; ++++ = 30–90 per cent.

^b Germination ++++ after being replaced in darkness for 2 days.

spores germinated in 2 to 3 days under the former conditions but very few in the dark. Similar results were obtained with spores of *P. helianthi* (Dec. 1, 1925; Dec. 9, 1925; Feb. 25, 1926; Apr. 24, 1926), many cultures having been tested. Moreover, with all three kinds of rusts, darkness proved less favorable than alternate light and darkness for the production of sporidia. A very large number of germination tests with teliospores of *U. caryophyllinus* was made. Generally a few spores germinated in alternate light and darkness in 6 to 8 days and a large number in 3 to 4 weeks; but sometimes they germinated better in continuous darkness. However, if, after the cultures had been kept in alternate light and darkness for 2 to 9 days, they were placed in continuous darkness, a high percentage ($50 \pm$) of spores always germinated in two days. Cultures kept in continuous darkness for a week generally failed to germinate; but if they were then kept in alternate light and darkness for two days and replaced in the dark, a very large proportion of spores germinated in two more days. Some results of these tests are presented in table 2.

OVERWINTERING OF TELIOSPORES OF PUCCINIA EMILIAE AND OF PUCCINIA XANTHII

In November, 1925, *Puccinia emiliae* was found in considerable abundance on calendulas in the greenhouses of the Department of Horticulture at the University of Missouri. It is not known how the rust was introduced. The calendulas were grown from seeds. This is apparently the first record of the occurrence of this rust in Missouri, although it had previously been reported from Indiana, Illinois, Kansas and Nebraska. Since teliospores of *P. emiliae* and of *P. xanthii* require no rest period before germinating, tests were made at intervals to determine how long they would continue to germinate. Collections of *P. emiliae* (Nov. 17, Nov. 27, and Dec. 1, 1925) and of *P. xanthii* (Oct. 1, 1925) were kept in a room that was cool during the winter but quite warm part of the time after May. Spores of *P. emiliae* were also kept in an ice chest. In all tests excepting the first, pieces of host tissue bearing spores were floated on distilled water. The results are given in table 3.

It is evident that teliospores of these two rusts may live over winter or longer under varying conditions of temperature and humidity as well as, in the case of *P. emiliae*, under rather uniform conditions in an ice chest. Taubenhaus (6) collected leaves of hollyhock bearing teliospores of *Puccinia malvacearum* November 26, 1908, and kept them in wire baskets at a low temperature both indoors and outdoors. The teliospores germinated from December 2 until April 10. He concludes that this rust may hibernate as teliospores on seeds. It is not impossible that *P. emiliae* may have been introduced into the greenhouses at Columbia as teliospores on seeds. It

TABLE 3.—*The viability of teliospores of Puccinia emiliae and Puccinia xanthii as measured by germination tests*

Rust species	Date of collections, 1925	Dates tested	Germination ^a
<i>Puccinia emiliae</i>	Nov. 17; kept in ice chest	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	++
do	Nov. 17	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, June 7, 1926	++++
do	Nov. 27	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+
do	Dec. 1	Dec. 19, 1925; Jan. 13, Feb. 10, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	+
<i>Puccinia xanthii</i>	Oct. 1	Oct. 1, Nov. 27, Dec. 19, 1925; Jan. 13, Feb. 10, Feb. 23, Mar. 2, Apr. 5, May 3, May 22, 1926	++++
		June 7, 1926	+++
		Oct. 1, 1926	0

^a 0 = no germination; + = less than 1 per cent; ++ = 1-5 per cent; +++ = 10 ± per cent; ++++ = 30 to 90 per cent.

disappeared in our greenhouses about midwinter but developed again in October, 1926.

EFFECT OF POTASSIUM DIHYDROGEN PHOSPHATE ON GERMINATION

In various tests, teliospores of certain rusts germinated better in a solution of KH_2PO_4 than in distilled water. Spores of *Uromyces spermacoces* collected December 14, 1924, were tested April 14, 1925. After eight days only a few spores had germinated in distilled water, but 5 to 10 per cent had germinated in 0.5 per cent KH_2PO_4 (table 1). In a test with spores of *U. caryophyllinus* April 7, 1925, in alternate light and darkness, after fifteen days only a few had germinated in distilled water but 20 to 50 per cent

had germinated in 0.5 per cent KH_2PO_4 (table 1). Similar results had previously been reported (5) for spores of *Puccinia helianthi* and have been obtained repeatedly since in solutions of 0.2 to 0.5 per cent KH_2PO_4 and in distilled water.

GERMINATION OF TELIOSPORES OF PUCCINIA HELIANTHI IN SOLUTIONS OF VARIOUS CHEMICALS

Germination of teliospores of sunflower rust was tested in solutions of hydrogen peroxide, chloroform, ether, dextrose, inulose, sucrose, ammonium nitrate, potassium nitrate, calcium nitrate, potassium permanganate, sodium chloride and combinations of some of these. Of course with organic compounds such as sugars, on account of the development of contaminating organisms, it was necessary to use spores that would germinate freely in a few hours. Spores germinated normally, apparently, in concentrations as high as 0.1 per cent NH_4NO_3 , 0.25 per cent KNO_3 , 0.125 per cent NaCl , 1.0 per cent $\text{Ca}(\text{NO}_3)_2$, 0.5 per cent KH_2PO_4 , 1.0 per cent maltose and sucrose and 2.0 per cent dextrose; but more or less abnormally in higher concentrations of these chemicals. Teliospores floated on distilled water to which 1 or 2 drops of chloroform or ether per 10 cc. of water had been added germinated about as well as in distilled water. Larger quantities of these chemicals partially or completely inhibited germination. Likewise, exposure of wet spores on leaves to vapor of ether (1-15 drops to 50 cubic inches of space) for 1 to 48 hours did not improve germination. When hydrogen peroxide was added to cultures (2 drops to 10 cc. of water), spores sometimes germinated more readily than in water, but these results could not be consistently duplicated. Pre-soaking spores for 24 hours in a 1 or 2 per cent solution of KMnO_4 did not increase the percentage of germination over that in the checks. It is evident therefore that, with the exception of KH_2PO_4 , the chemicals tested had little or no favorable effect on germination.

TELIOPORES THAT FAILED TO GERMINATE

Attempts to germinate the teliospores of the following rusts have been unsuccessful, in some cases probably because the incubation period was too short. For the first five species this period was approximately one month and for the last three approximately two months. The months when the tests were started are indicated after each species, and in all cases the spores were of the current season.

<i>Puccinia amorphae</i> Curt. on <i>Amorpha fruticosa</i> L.	Oct. and Jan.
<i>P. bullata</i> (Pers.) Wint. on <i>Taenidia integerrima</i> (L.) Drude	May
<i>P. coronata</i> Corda on <i>Avena sativa</i> L.	Jan.

<i>P. osmorhizae</i> (Peck) Cke. and Pk. on <i>Osmorhiza</i>	
<i>longistylis</i> (Torr.) DC.	May
<i>P. podophylli</i> Schw. on <i>Podophyllum peltatum</i> L.	May
<i>P. bardanae</i> Corda on <i>Arctium minus</i> Bernh.	Oct.
<i>P. graminis</i> Pers. on <i>Triticum vulgare</i> Vill.	Oct.
<i>P. pruni-spinosae</i> Pers. on <i>Prunus persica</i> (L.) Stokes	Nov.
and on <i>Prunus scrotina</i> Ehrh.	Oct.

SUMMARY

The principal results of these and my previous observations on germination of teliospores of nearly thirty species of rusts may be summarized as follows:

1. If spores are kept alternately wet and dry, or floated continuously on distilled water at room temperature in alternate light and darkness, some generally germinate in 2 to 6 weeks.

2. As the season advances there is a decrease in the time necessary for germination to begin and for complete germination (highest possible percentage).

3. Treatment with various chemicals, except to adjust the H-ion concentration, had little or no favorable effect. However, spores may germinate in relatively high concentrations of certain chemicals.

4. Spores of *Puccinia helianthi*, *P. emiliae* and *P. xanthii* germinated more readily and produced many more sporidia in alternate light and darkness than in continuous darkness.

5. Spores of *Uromyces caryophyllinus* germinated very readily if cultures were kept in alternate light and darkness for two to several days and were then placed in continuous darkness. Even in continuous darkness germination sometimes occurred, and in such cases in less time and more profusely than in alternate light and darkness.

6. Spores of some rusts formed late in the growing season apparently germinate more readily than those formed earlier.

7. Under certain conditions spores of *Puccinia emiliae* and of *P. xanthii*, although they require no rest period, may continue to germinate after being stored over winter or longer (8 to 10 months).

8. Attempts to germinate the teliospores of several species of rusts, collected soon after spore formation, have never been successful, in some cases probably because the incubation period has been too short.

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SOME CHEMICAL TREATMENTS OF SOIL FOR THE CONTROL OF DAMPING-OFF FUNGI

H. E. THOMAS

Seedlings grown in soils treated with steam or formaldehyde are without protection against subsequent introduction of pathogenic fungi. The expense and other factors render these methods of treatment slow of adoption. There is therefore an urgent need for fungicides effective in concentrations that may safely be used in association with the underground parts of growing plants and that may afford continuous protection.

It is apparent that damping-off cannot be approached as a general problem, since marked specificity of pathogens for susceptibles and of chemicals for both pathogens and susceptibles is well known. This paper is concerned chiefly with the damping-off of tomatoes and cabbage in the greenhouse.

PREVIOUS WORK

Although some success was reported in the chemical treatment of soils by early workers, among them Halsted (9) in 1900, it is in the last decade that more definite progress in this direction has been made. Bewley (3) has made extensive tests of copper compounds and other chemicals in the soils of glasshouses. His Cheshunt mixture (2) proved effective in the control of damping-off of tomato and other plants when both *Phytophthora* and *Rhizoctonia* were apparently involved. Gratz (8) tested this mixture for *Rhizoctonia* of cabbage with discouraging results. Sherbakoff (14) used copper sulfate for *Rhizoctonia* of lettuce with some success. Gloyer and Glasgow (7) and Clayton (4) have successfully used mercury compounds for rhizoctonia, damping-off, and other diseases of crucifers. Monteith and Harmon (13) found mercurous chloride to be the most satisfactory of many chemicals tested in the control of brown patch of turf caused by *Rhizoctonia* spp. Major (12) found that tobacco plants were injured by mercury compounds when applied in sufficient concentration to control *Thielavia* damping-off. Hartley (10) and Wiant (15) have successfully employed sulfuric acid, aluminum sulfate, and various compounds of mercury for the control of damping-off of coniferous seedlings. Much has been written concerning the beneficial effect of chemicals upon seedlings aside from disease control. It seems probable that a considerable part of this apparent stimulation may be accounted for, however, by the control of pathogenic fungi (1).

MATERIALS AND METHODS

The fungi employed in these experiments were two strains of *Phytophthora*, isolated from tomato; and three strains of *Corticium vagum*

B. and C., isolated from cabbage, beet, and pepper. The soil was a rather heavy clay loam used mostly in flats approximately 18 x 18 x 2 inches. Inoculations were made in most cases from cultures of the fungus grown on steamed wheat kernels. Occasionally, infested soil was used. A known quantity of seed (500 seeds per flat in nearly all cases) was used. Unless otherwise indicated, treatments were made immediately after planting. The chief chemicals employed were copper carbonate, copper sulfate, mercuric chloride, and chlorophenol mercury.

EXPERIMENTS WITH TOMATOES

In several preliminary trials with tomatoes, the Cheshunt mixture (2) and copper carbonate gave similar and substantial increases in total and disease-free seedlings on soil inoculated with *Phytophthora*. In subsequent tests, copper carbonate was used alone or in comparison with mercury compounds, and occasionally with other chemicals.

Treatments at planting time.—In table 1 are summarized the results of treating tomatoes at the time of planting on soil inoculated with *Phytophthora*. The varieties Ponderosa and John Baer were used. *Rhizoctonia* from cabbage and *Botrytis* from lettuce did not influence the stand of tomato seedlings.

TABLE 1.—*The effect of treating tomatoes at planting time on Phytophthora-infested soil*

Treatment	No. plants surviving in 7 experiments ^a						
	1	2	3	4	5	6	7
Copper carbonate 2 gm.	371						
do 3 gm.		357					
do 5 gm.			214			302	269
			292			274	284
Corona copper carbonate 15 gm. ...			221	224	224		
			223	282	237		
Colloidal copper							316
CuSO ₄ 3 gm.—1000 cc. H ₂ O							229
HgCl ₂ 0.5 gm.—1000 cc. H ₂ O						364	
HgCl ₂ 0.5 gm.—800 cc. H ₂ O							244
HgCl ₂ 1 gm.—1600 cc. H ₂ O							115
Uspulun 2 gm.—1000 cc. H ₂ O						338	
Uspulun 2 gm.—800 cc. H ₂ O							273
Uspulun 4 gm.—1600 cc. H ₂ O							138
Check	227	102	145	91	65	334	302
Check			136	101	67	215	193

^a Each number represents the plants produced in a flat planted with 500 seeds.

The duration of the experiments was variable, extending over periods as long as 47 and 49 days from the time of planting to the taking of final notes. Corona copper carbonate with copper content approximately equivalent to five grams of undiluted copper carbonate was used, and gave essentially similar results. Strengths at least double those employed here have been used on tomato without appreciable injury. For convenience in application, the insoluble carbonates, as well as the soluble chemicals, were applied in water. The average number of surviving plants in all copper carbonate flats was 269.5, and in the corresponding checks 164.8, out of a total of 500 seeds sown. While the higher concentrations of the mercury compounds caused obvious injury, the lower concentrations offer considerable promise for further testing.

Treatments after planting time.—It is obvious that a method of controlling damping-off involving treatments begun after the disease appears among the seedlings is very desirable. Four experiments were made with this possibility in mind. No marked or consistent benefit was derived from applications of copper carbonate made after damping-off had appeared. In one case an application made six days after planting was quite as effective as were those made at planting time, but no disease was noted in these flats until nine days after planting. It seems probable, however, that late treatments might be beneficial to large areas in which localized infestations appear.

Experiments with tomatoes on soil inoculated with Rhizoctonia.—In several earlier experiments, the *Rhizoctonia* from cabbage produced no appreciable damping-off of tomatoes. Later, in a single experiment, *Rhizoctonia* from beet produced only slight injury. Still later a strain isolated from pepper seedlings was tested on pepper, tomato, and cabbage in comparison with the cabbage strain. The pepper strain was pathogenic to pepper and tomato but not to cabbage. The cabbage strain produced severe damping-off on cabbage and little or no disease on pepper or tomato. Tomatoes were then planted in four flats inoculated with the pepper strain, and three of these were treated respectively with mercuric chloride one-half gram, mercuric chloride one gram, and Uspulun two grams, each in one liter of water. The fourth served as a check. Final counts on these flats were respectively 329, 68 (chemical injury), 395, and 88. In another similar experiment of eight flats, copper carbonate (10 grams) and copper sulfate (3 and 6 grams) produced as good stands as the weaker mercury compounds, but the loss in the check was much less severe. The stronger mercuric chloride (1 gram) and Uspulun (4 grams) again caused serious injury.

Again 10 flats were similarly treated, with the following results: mercuric chloride (0.5 gram), 371 and 341; copper sulfate (6 grams), 97 and

115; copper carbonate (5 grams), 95 and 181; Corona colloidal copper (not measured), 25 and 54; checks, 13 and 30 plants per flat. Copper sulfate caused marked retardation of the seedlings in this experiment but did not prevent serious damping-off. It is apparent that under these conditions of severe damping-off the copper compounds are not effective in controlling *Rhizoctonia*.

Distribution of chemicals in soil.—It has been shown that copper compounds are very quickly taken out of suspension or solution when added to soils (5, 11). It seemed, therefore, that these might profitably be introduced into the soil to greater depth than would be reached by the methods employed in the earlier part of this work. A one per cent solution of sodium chloride with a one-half per cent solution of copper sulfate was first used (one liter per flat), in four flats inoculated with *Phytophthora*. Although the concentration and quantity of chemicals was much lower than those used by Hunt and his colleagues (11), there was a marked retardation in germination and early development of the seedlings. These flats, however, produced substantially greater numbers of seedlings than untreated flats. The same concentration of sodium chloride was then tested alone and in combination with Corona copper carbonate, 15 grams per flat, on *Phytophthora*-inoculated flats. Emergence of seedlings was retarded one to two days by salt alone, and four days by salt plus copper carbonate. Total seedling counts were highest on the copper carbonate flats, lowest on the salt flats, and intermediate on untreated flats (averages 330, 272, and 293 respectively).

In two experiments copper carbonate at double the quantity generally used for surface treatments was thoroughly mixed with the soil by means of a barrel churn. In both instances following inoculation there were only small increases in numbers of seedlings over those on untreated soil. In one of these, in flats given the usual surface treatment, there were twice as many plants as in the checks. It seems, therefore, that under these conditions the concentration of chemicals near the surface of the soil is more desirable than vertical distribution through the soil.

Chemical injury to tomatoes.—Copper carbonate, when used alone in the quantities usually employed here, has not produced injury whether applied at planting time or on the growing plants, with one exception, in which the plants were somewhat retarded. Heavier applications have occasionally caused a gradual yellowing of plants after several weeks. Copper sulfate at six grams per flat sometimes caused considerable retardation in growth of seedlings.

The tomato is more sensitive to injury by mercury compounds than is cabbage. Applications of these chemicals at planting time may either reduce the percentage of germination or merely retard the rate of growth of

seedlings. Mercuric chloride (1-1600) or Uspulun (1-400) applied to the growing plants kill areas on the foliage and injure the stems; the injury is most pronounced slightly above the ground level. Injury in these experiments has usually been more marked with mercuric chloride than with Uspulun when the materials were used in the proportions given above. Since this is the only appreciable difference noted between these chemicals, the figures from five trials bearing on this point are assembled in table 2. The soil had been inoculated with a damping-off fungus in all of these cases except one, but the consistently high counts of seedlings in flats in which lower concentrations of chemicals were used are sufficient practically to eliminate damping-off from consideration in comparing the results of heavier applications. It is believed that variations in the temperatures of the greenhouses and of the solutions employed has an important bearing on the amount of injury produced. Gassner (6) has shown that even with seed treated at the same temperature, a marked variation in injury follows when the seeds are germinated at different temperatures.

TABLE 2.—*The effect of mercuric chloride and Uspulun on stands of tomato seedlings when applied at planting time*

Treatments	No. plants surviving in 5 experiments ^a				
	1	2	3	4	5
HgCl ₂ - 0.5 gm. - 1000 cc. H ₂ O		315	329		364
do - 800 do	244				
HgCl ₂ - 1 gm. - 1600 do	115				
do - 1000 do		74	68	373	
Uspulun - 2 gm. - 1000 do		314	395		338
do - 800 do	273				
Uspulun - 4 gm. - 1600 do	138				
do - 1000 do		196		406	

^a Each number represents the plants produced in a flat planted with 500 seed.

EXPERIMENTS WITH RHIZOCTONIA OF CABBAGE

Since *Rhizoctonia* in the soil is one of the fungi especially resistant to chemical treatment, several experiments were made in the control of this fungus with cabbage as the test plant. The results of seven such tests are shown in table 3. All figures included here represent results in flats except those in the last column, which were obtained in a greenhouse bed. The Corona colloidal copper was peppered rather heavily on wet soil and was not measured. In general, mercuric chloride and Uspulun are effective in the control of this fungus; whereas the copper compounds are relatively ineffective. At times, however, in treated flats, "wire stem" (8) appeared

and some seedlings were dead before the final counts were made, indicating that a second application would be necessary in some cases. Delayed single applications, as in the tests on tomatoes, were not effective. The slight injury caused occasionally by mercury compounds was not greater in the case of corrosive sublimate than that caused by chlorophenol-mercury.

TABLE 3.—*The effect of treating cabbage at planting time on Rhizoctonia infested-soil*

Treatment	No. seedlings surviving in 7 experiments ^a						
	1	2	3	4	5	6	7
HgCl ₂ - 0.5 gm. - 1000 cc. H ₂ O				296	254		
do 12 days after planting					146		
HgCl ₂ - 1 gm. - 1000 cc. H ₂ O					301		
do - 1600 do						216	
Uspulun - 2 gm. - 1000 do				346	240		
do 12 days after planting					160		
Uspulun - 2 gm. - 800 cc. H ₂ O			221				
do - 3 gm. - 1000 do	260						
do - 4 gm. - 1600 do						222	
do - 4 gm. - 1000 do					268		
do - 4 gm. - 800 do			273				
do - 5 gm. - 1000 do 37 days before planting		169					
do - 2.6 gm. per sq. ft.							344
Copper carbonate - 1 gm. per sq. ft.							17
Corona copper carbonate - 30 gm.			34				
Colloidal copper Corona						40	
Colloidal copper - 2 gm. - 1000 cc. H ₂ O			9				
Colloidal copper - 4 gm. - 1000 cc. H ₂ O			12				
CuSO ₄ (crystal) - 3 gm. 1000 cc. H ₂ O					171		
do - 6 gm. do					241		
Check	34	51	0	111	181	8	1
Check					165		

^a Each count represents the seedlings produced from 500 seeds.

OTHER OBSERVATIONS

The dictum that "all general statements are in error including this one" finds ready application in the consideration of damping-off diseases, the problems of which are multiplied by each kind of soil, pathogene, suscept, and chemical involved.

The conditions imposed here were, at least in many cases, more severe than those of the usual greenhouse practice. In some experiments a considerable number of the seedlings of check flats never appeared above ground. Thus a phenomenal difference in total seedlings may be recorded

for treated and untreated flats. It has been recognized more recently that this condition has been mistaken for chemical stimulation (1).

A marked difference is noted in these experiments in the action of different chemicals upon various plants as well as upon the different fungi. For example, copper carbonate was effective in controlling *Phytophthora* of tomato and apparently *Botrytis* sp. of lettuce, but almost completely ineffective in control of *Rhizoctonia* of cabbage. The same material caused no injury to cabbage, tomato, cucumber, lettuce and beets, but was found by Wiant (15) to cause severe injury to conifers. Uspulun used in concentrations that caused no apparent injury to cabbage was injurious to bean, cucumber, tomato, and lettuce. Tobacco appears to be more sensitive to both copper and mercury compounds in the soil than was any other plant studied.

In several experiments, plantings of tomato and cabbage were treated with copper and mercury compounds with the primary purpose of studying chemical stimulation of the seedlings. Neither in these nor in other experiments has there been consistent evidence of increase in size of plants on treated flats. Increases in numbers of seedlings, whenever this occurred, is more reasonably explainable on the basis of disease control.

SUMMARY

Copper carbonate, mercuric chloride, and Uspulun controlled damping-off of tomatoes caused by *Phytophthora* spp.

The mercury compounds caused injury to tomatoes in concentrations which did not injure cabbage.

The mercury compounds were effective in controlling *Rhizoctonia* in cabbage and tomato plantings; whereas copper carbonate and two forms of colloidal copper were almost completely ineffective.

Treatments after damping-off had appeared were of little value to tomatoes or cabbage.

There was no evidence of chemical stimulation in any of these experiments.

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A BACTERIAL DISEASE OF BOWLESIA

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Bowlesia septentrionalis Coulter and Rose is an annual caulescent herb belonging to the Ammiaceae. It occurs in rich soil or shaded places throughout the Southwest from Texas to California. Plants begin to appear during December, reach the maximum abundance during April and May, and disappear as the hot, dry weather approaches.

During several seasons a bacterial disease has been noted frequently on plants growing in the vicinity of Austin, Texas. Symptoms appear on the leaf blades and petioles shortly after the leaves unfold and, as the growing season advances, the infection becomes so general that uninfected plants are rare. The diseased leaves show numerous characteristic water-soaked spots which very soon become almost black. These spots are frequently marginal but not always so. With age they become somewhat irregular in shape and the tissue dries out, becoming reddish brown. When the petiole is infected, the entire leaf droops and withers. It often happens that entire lobes of the leaf are killed (Fig. 1). The youngest spots have the appearance of a bacterial disease. That they are caused by one of the green fluorescent species of plant-pathogenic bacteria has been proved by isolation of the organism and subsequent inoculation of healthy plants with pure cultures.

For purposes of isolation, young spots from the first infected leaves which appeared in January were chosen. The leaves were rinsed through several changes of sterile water but were not disinfected. The spots were removed with sterile instruments and thoroughly crushed in a few drops of sterile broth. A single loopful of this inoculum was then spread over the surface of an agar plate by means of the loop needle. After 24 hours incubation at 27° C. the colonies are visible but quite small. They increase in size and reach the maximum after about 48 hours incubation. On agar plates prepared in the above manner numerous colonies develop. In all of the plates prepared for isolation there was a single predominating type of colony. Occasional contaminations with soil bacteria occur, but such colonies are invariably few in number and not easily mistaken for the true causal organism. Single, well isolated colonies were transferred to plain agar slants. The cultures so established were subsequently studied as to cultural reactions, morphology, and physiological and pathogenic properties.

CULTURAL FEATURES

Cultures in all media except gelatin were incubated at 27° C. The reaction of the culture media was adjusted to pH 7 unless otherwise stated.

Agar Plates. On the surface of agar plates the colonies reach a diameter of 5-7 millimeters. They are smooth, moist and glistening, somewhat yellowish with entire margin. On beef infusion agar a beautiful greenish pigment is produced which diffuses throughout the agar. The growth is quite viscid when touched with the needle.



FIG. 1. Bacterial spots on leaves of *Rowlesia septentrionalis*.

Agar Slant. On the sloped surface of agar inoculated with a straight needle the growth becomes evident as a fine delicate line at the end of 24 hours. In 48-hour cultures the growth has attained a width of 2-4 mm. with smooth or slightly undulate margin. It is yellowish, moist, glistening

and viscid. The green fluorescent pigment is produced abundantly on infusion agar. After a few days the growth changes from a copious viscid mass to a thin film. This feature is quite characteristic of all the agar cultures.

Beef Infusion Broth. In young cultures the broth is uniformly cloudy throughout the medium. As the culture becomes older, a heavy viscid sediment forms at the bottom while small zoogloal masses occur at the surface. The broth becomes greenish in cultures five days old.

Gelatin. The liquefaction of gelatin occurs promptly. In cultures 48 hours old there is a napiform area of liquefaction. By the end of seven days the process of liquefaction is completed.

Steamed Potato. The organism grows well on the sloped surface of steamed potatoes. In old cultures there is a heavy yellow slime covering the entire surface.

Steamed Sweet Potato. Growth is copious on this medium. It is more yellowish than growth on agar.

Litmus Milk. Coagulation of milk occurs in 48 hours. The reaction becomes alkaline. Digestion of the protein begins after the second day and progresses rather slowly. In cultures seven days old there is a heavy viscid mass at the bottom with some solid particles floating at the surface. The litmus is not reduced.

Coagulated Blood Serum. Locflier's blood serum medium becomes covered with a slimy yellowish growth. Liquefaction occurs slowly.

Crowe's Medium. On a medium described by Crowe consisting of one part glucose agar and three parts of defibrinated blood the growth is moderately heavy with a greenish color.

Sugar Agars. On plain extract agar containing 1 per cent glucose, sucrose, maltose or lactose and 1 per cent of Andrades indicator the growth is best at the top but extends along the line of inoculation to the bottom of the tube. Acid is produced from glucose and maltose.

Cohn's Solution. Growth occurs in Cohn's solution producing a uniform turbidity. The growth is not, however, abundant.

Fermi's Solution. Growth is similar to that on Cohn's medium.

Uchinsky's Solution. The growth begins promptly and with much greater vigor than in the other synthetic media.

Ferric Ammonium Citrate Agar. On the sloped surface of synthetic agar containing 0.8 per cent of the citrate as the sole source of carbon, growth is vigorous. A rusty red deposit of iron hydroxide occurs on the surface, indicating that the citrate is utilized.

MORPHOLOGY

The organism is rod-like, straight with rounded ends, borne singly or sometimes in pairs and short chains. It stains readily with aqueous solu-

tions of gentian violet, fuchsin or methylene blue. When stained by Gram's method the gentian violet is not retained. Capsules have not been demonstrated. Cultures grown on a variety of media were stained by both the Hiss and Ribbert's methods. Young cultures are very actively motile by means of bipolar flagella. The flagella have been stained by means of Loeffler's, Casares-Gil's and Gray's methods. They occur only at the poles and are generally two or three in number at each end of the cell. They are slender, flexuous, about three times the length of the rod. Spores have not been shown either in stained or unstained mounts. Involution forms, if present, are never prominent. In fresh young cultures grown on beef infusion agar and stained with aqueous gentian violet, the rods measure 1.2 to 1.6 microns in length by 0.5 to 0.7 microns in diameter. Color granules have not been observed.

PHYSIOLOGICAL CHARACTERISTICS

Thermal Relations. The optimum temperature for growth is about 27° C. Growth does not occur at 37° C. nor at -1° C. Below 5° C. development takes place very slowly. That the organism is capable of developing at rather low temperatures is evident from the fact that the disease is widely spread during the cool winter months when the temperature varies from about 20 to 75° F. The thermal death point for 48-hour cultures in neutral broth is 49° C.

Resistance to Desiccation. The ability to survive desiccation was tested by placing drops of a 48-hour broth culture on sterile cover slips and transferring one of these to sterile broth at daily intervals until no growth was obtained. Under this condition, the organism has little resistance to desiccation, as growth failed at the end of four days. It is evident, however, that under natural conditions it must be able to survive much longer in the dry summer months.

Oxygen Relations. In fermentation tubes containing various sugars or other fermentable carbon compounds, growth occurs only in the open arm of the tube. Anaerobic cultures fail to grow. The technique used was that of oxygen absorption by means of pyrogallie acid and potassium hydroxide.

Relation to Reaction of Medium. The organism grows through a rather wide range of H-ion concentration. The limits for growth on plain extract peptone agar appear to be about pH 4.5 and 8.6 with the optimum growth at pH 7.2. In 1 per cent peptone beef extract broth the reaction was changed from an initial pH 7.1 to pH 8.6.

Fermentation. The ability to ferment various carbon compounds was tested in neutral 1 per cent peptone beef extract broth plus brom thymol blue indicator. Acid, but no gas, was produced in glucose, maltose and xylose. No fermentation occurred in sucrose, lactose, arabinose, mannite,

galactose, levulose, cellobiose or inulin. The experiment was continued for 24 days. At the end of this time the xylose and maltose broth had become alkaline, although the glucose remained acid.

Indol Production. Cultures were grown seven days in tryptophane broth and tested for indol by Ehrlich's method. Indol is produced.

Production of Hydrogen Sulphide. The test for hydrogen sulphide was made by culturing the organism in beef extract agar plus lead acetate. A blackening of the agar occurred promptly, indicating the presence of hydrogen sulphide.

Reduction of Nitrates. Cultures were prepared in nitrate broth and tested for nitrites by the sulphanilic acid and naphthylamine hydrochloride test. Nitrates are reduced to nitrites.

Ammonia Production. Cultures grown in beef extract peptone broth and tested with Nessler's reagent show the presence of ammonia.

Reduction of Methylene Blue. The dye is reduced in peptone broth cultures.

BIOLOGICAL RELATIONS

The organism is pathogenic to *Bowlesia septentrionalis*. Plants sprayed with a water suspension prepared from an agar culture and kept in a moist atmosphere produced typical spots within five days. Leaves which were punctured with a needle and inoculated by placing a small bit of an agar culture in the wounded tissue developed enlarged spots which contrasted sharply with uninoculated punctures. Similar methods of inoculation on other plants gave no positive results. Other species growing near *Bowlesia* in the field are not infected so far as is known.

TECHNICAL DESCRIPTION

This organism does not appear to agree in all particulars with any of the yellow fluorescent plant pathogens which have been described. It is therefore considered as a new species in the genus *Phytomonas*.

Phytomonas bowlesii n. sp.

A short rod with rounded ends, borne singly, in pairs or very short chains; actively motile by means of bipolar flagella which are two to five in number at each end of the rod; no spores or capsules distinguished; produces green fluorescent pigment; liquefies gelatin and blood serum; coagulates milk and digests the casein; does not reduce litmus; reduces methylene blue and nitrates; produces hydrogen sulphide, ammonia, and indol; grows in Cohn's, Uschinsky's and Fermi's solutions; utilizes ferric ammonium citrate and deposits iron hydroxide; optimum temperature 27° C., minimum

temperature -1°C. , maximum temperature 35°C. ; thermal death point 49°C. ; resists desiccation four days; stains with basic analine dyes; not acid fast, negative to Gram's method of staining; optimum reaction of medium pH 7.2, minimum pH 4.5, maximum pH 8.6, pathogenic to *Bowlesia septentrionalis*.

SUMMARY

A new bacterial disease of *Bowlesia septentrionalis* is reported. By means of isolations and inoculations, the causal organism has been shown to be a new species of the genus *Phytomonas*. The cultural features, physiological and biological properties, and the morphological characteristics of the organism are given.

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COMMERCIAL TOBACCOS AND CURED LEAF AS A SOURCE OF MOSAIC DISEASE IN TOBACCO ¹

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The question of the source of the initial mosaic infections of tobacco in the plant bed and the field has not been satisfactorily answered, nor has the source been ascertained of sporadic cases of the disease in experimental and commercial greenhouses, where tomatoes and other susceptible plants are being grown. Weed hosts are usually quite prevalent in tobacco sections of Kentucky and may be considered an important source of infection. However, when they are completely removed from a plant bed and plants from the bed are set in the usual manner, extensive infection may be evident soon after growth commences. Because tobacco plants are handled so much in the operations of weeding, transplanting, worming, suckering and topping, the personal habits of the workers, with respect to the use of tobacco, should be given careful consideration in their relation to the introduction and spread of the disease.

The following studies were made with the object of determining to what extent the use of tobacco by workmen might be responsible for at least a part of the mosaic infection in the plant bed and the initial infection in the field, and they may serve to explain many cases of mosaic infection in greenhouses where an obvious source of infection did not appear to be present.

After several seasons of observation of plant beds on the Experiment Station farm, we are of the opinion that mosaic rarely if ever develops in plant beds which have been sowed and then not touched by man; whereas in beds which have been weeded by the average farm hand the disease is likely to be found on an occasional plant and following the first pulling may be widely distributed in the bed. This might be explained on the basis of the presence of occasional weed carriers, but from the results obtained where care has been used not to introduce the disease on the hands of the workers, it would seem that man may be as important a source of infection

¹ Published with the approval of the Director of the Kentucky Agricultural Experiment Station.

as the weed hosts. For example: in a rotation, one field of which is set to about 13,000 tobacco plants each year, records have been kept of the initial mosaic infection since 1922. In 1922, 1923, and 1924, with no precautions other than pulling suspicious weeds from the plant bed some time before pulling plants, the mosaic was 9 per cent, 6 per cent, and 8.7 per cent, respectively, after growth commenced following transplanting. In each of these years men pulling plants used "natural leaf" chewing tobacco. In 1925 the mosaic infection was only 2.1 per cent; two of the men who pulled plants used no tobacco, while one chewed plug tobacco. This precaution was taken to prevent possible angular leaf-spot infection from entering the bed from "natural leaf" chewing tobacco. Toward the end of the planting one of the men who had not chewed was given permission to chew five-year-old "natural leaf" tobacco, and following this the initial mosaic infection in the field rose to 16 per cent. Subsequent tests of this old tobacco indicated that it carried mosaic, as 100 per cent of the plants inoculated with a decoction of it developed mosaic. In 1926 the men who pulled and set were furnished with "natural leaf" and plug tobacco sterilized in the autoclave with the object of destroying both leaf-spot organisms and mosaic. The initial mosaic infection in the field was 0.44 per cent.² Three weeks later no mosaic was found in the bed from which these plants were pulled. One end of this bed was located within a few feet of the tobacco field where tobacco had been growing in rotation for several years, thus offering a good opportunity for the weed hosts in the neighborhood to become infected. Bull nettles and ground cherries are very prevalent all about this field. The results suggest that the weed hosts have not been an important factor in initial infection in the years when infection was high.

Another bed on the Experiment Station farm, in which 166 different strains of tobacco were grown and set in the field, was found to be free from mosaic at setting time and three weeks later. Particular care was taken to prevent the introduction of any mosaic on the hands: the plants were pulled by the writers, who washed their hands before each lot of plants was pulled. In spite of these precautions, 0.44 per cent infection resulted in one field of 3,000 plants, and 0.016 in another of 5,500 plants. There was no infection in a third field of about 2,000 plants. Each field had been used for tobacco continuously. From the same bed a block of 20,800 plants from 53 separate pullings (variety test) had developed 0.13 per cent mosaic 25 days after setting; a block of 2,000, 1.05 per cent, and another block of 3,700 plants was entirely free from mosaic. In all cases except the one in which 1.05 per cent mosaic developed, the infected plants were scattered

² In 1927, under the same conditions, the initial infection in 13,287 plants was .06 per cent.

and infection appeared not to be associated with pulling. In the one case infection was largely limited to two varieties and might have been associated with pulling. The source of these infections is not known. Six isolated beds of plants in a wood were still free from mosaic in the fall, and ten isolated plots of tobacco set from them by the writers were free from infection with the exception of one plant which grew within a few inches of a diseased ground cherry. These plants were set in a field which had not grown tobacco for many years.

At the western Kentucky substation, where the men were not allowed to use any tobacco while setting and washed their hands before handling plants, no mosaic had developed in extensive plantings several weeks after setting. In experimental plots where plants were pulled and set by men using "natural leaf" chewing tobacco, the mosaic infection was 33.8 per cent; in alternating check plots pulled and set by men with clean hands and not using tobacco, the infection was 9 per cent. The counts were not made until July 19, when the plants were more than knee high. Cultivation had been across the plots, giving an opportunity for spread from row to row. These results, although by no means conclusive, suggest strongly that the human factor is an important one in initial mosaic infection, and suggest that, by eliminating as nearly as possible the virus from the hands of workers, initial mosaic infection may be greatly reduced.

Clinton³ showed several years ago that if tobacco trash were used as a plant bed fertilizer, mosaic might be expected to develop. The idea, therefore, of cured tobacco acting as a source of infection is not new. Tests of random samples of cured Burley tobacco show that they are usually capable of causing infection in healthy plants.

EFFECT OF AGE ON TOBACCO MOSAIC VIRUS

Age seems to have but little effect on the virus in cured tobacco as indicated by the five-year-old samples above mentioned. Further tests on the effect of age on the virus have been made with samples collected by the Chemistry Department of the Kentucky Experiment Station and carefully preserved in covered glass jars properly labeled. These were kindly furnished by Dr. A. M. Peter. These samples ranged in age from 15 to 30 years when first obtained by us. The early inoculations were made by wetting the ground tobacco with water and rubbing it on the leaves with the fingers. The hands were washed between each inoculation with soap and running water and nothing but the plant was subsequently touched, the decoction being poured on the hands of the operator by another person. In these tests of old material, positive results were obtained from material 15 to 16 years old, 17 to 18, 23, 26 to 27, and 29 to 30 years old.

³ Clinton, G. P. Report of the station botanist. Conn. Agr. Exp. Sta. 1914.

In a later test the plants were not touched at all with the hands, but each individual plant was inoculated with a separate decoction applied with individual swabs of cheesecloth on sticks, the leaf being held with a piece of waxed paper, changed for each plant. All implements including glassware and swabs were sterilized under high steam pressure before using or by boiling 15 minutes in water, leaving no apparent source of accidental infection. Several leaves on each plant were rubbed to increase the chance of infection. The sixth and seventh plants throughout the series were inoculated with the 31-year-old sample, steam sterilized 15 minutes at 10 pounds pressure, making 18 plants in all which were held as checks. These were placed at intervals between the other plants in the bench. Insects were kept in control by cyanide fumigation. The results of this test, in which there appeared to be no source of accidental infection, are given in table 1.

TABLE 1.—*The results of inoculating tobacco plants with decoctions from ground tobacco of different ages*

No. plants inoculated	Age of inoculum in years	No. plants infected	Incubation periods in days	Type of mosaic
6	16	1	13	Severe
6	17	2	13, 13	Not recorded
6	18	5	6, 8, 9, 9, 9	1 severe, 4 mild
6	20	6	8, 8, 9, 9, 9, 10	Mild
6	24	0
6	28	0
6	30	1	8	Mild
6	31	3	8, 13, 16	Mild
18	Mosaic tobacco sterilized	0

These results seem to show conclusively that tobacco samples kept in closed jars in a relatively dark room for periods of 16 to 31 years may still be viruliferous.

Two types of mosaic resulted from these inoculations. The majority of infections were of the mild type in which a mosaic pattern is clearly evident although the contrast in color is not great and the leaves are not distorted. There was also a more severe type in which the pattern is striking and the leaves usually distorted. These two types are commonly present in Kentucky tobacco fields. In one case (18-year-old inoculum) both types resulted. In all cases the inoculum used consisted of a composite of samples collected the same year, so the appearance of both types of mosaic is not surprising. The fact that the mild type predominated does not necessarily

mean that it is longer lived than the severe type but suggests that in selecting samples for analyses only those were taken which appeared healthy. As the more severe mosaic causes much more prominent symptoms than the mild, the chances of plants being selected which were infected with severe mosaic were comparatively small.

MOSAIC IN "NATURAL LEAF" CHEWING TOBACCO

Although it is by no means certain that all tobacco is infected with mosaic at cutting time, yet it may be assumed that following the operations of worming, suckering, and topping, comparatively few plants remain free from the disease. This assumption is borne out by observations of the disease in the second crop of suckers.

A large majority of Kentucky tobacco growers chew tobacco taken at random from the cured leaf and made into twists. As this tobacco receives no further treatment, it is almost certain that any of it may contain the mosaic virus. As a result of handling the tobacco in a dry state and of wiping the mouth while chewing, the hands are almost certain to become contaminated with the virus. Weeding and pulling plants for setting is usually done while the plants are wet, thus affording ideal conditions for inoculation of an occasional plant during these operations if the hands have become contaminated. Although the facts cited earlier in this paper indicate that this source of mosaic infection may be an important one, another example is cited based on an experiment with tomatoes conducted by the writers. A small portion of a steamed tobacco bed was sowed to tomatoes. Solanaceous weeds had been dug from the bed in previous years. On June 9, 1926, 28 tomato plants were pulled while tobacco was being chewed which was known to be viruliferous. The mouth was wiped from time to time during pulling so that the fingers became contaminated with saliva. These plants were set, the hands washed in soap and water, and 28 more plants pulled from the same bed and set in the next row. In 16 days, 21, or 75 per cent, of the plants pulled with contaminated hands were showing positive signs of mosaic, while the check plants were all healthy 36 days after setting.

MOSAIC IN COMMERCIAL TOBACCO

Considering the almost constant presence of mosaic in tobacco fields, and in view of the longevity of the virus in dry tobacco, commercial tobaccos must all be looked on with suspicion as possible carriers of mosaic. It is probable that a more extensive knowledge of the manufacturing processes used in the preparation of certain brands of tobacco will automatically eliminate them as possible carriers of the disease. For example, we have

TABLE 2.—*The effect of inoculating Turkish tobacco plants and tomatoes with decoctions of commercial brands of tobacco. The inoculations were made in the order given*

Brand ^a	No. plants inoculated	No. plants mosaic	Incubation periods in days
<i>Experiment I</i>			
Reynolds' Natural Leaf (1), plug	5	3	15, 17, 19
Yellow Tag, do	3	0
Apple Suncured (1), do	3	1	12
Old Kentucky, do	3	0
Penn's Red J., do	3	0
Star, do	3	0
King Pin, do	3	0
Futurity, twist	3	0
Brown's Mule (1), plug	3	1	17
Climax (1), do	3	3	13, 17, 23
Richland, twist	3	0
Strater's Natural Leaf, do	3	0
Drummond (1), plug	3	0
Hancock's Fig, do	3	0
Checks (Clean hands and water)	2	0
<i>Experiment II^b</i>			
Apple Suncured (1), plug	1	0
Old Kentucky, do	2	0
Penn's Red J., do	3	0
Star, do	3	0
Brown's Mule (1), do	2	0
Strater's Natural Leaf, do	3	0
Drummond (1), do	3	0
Sterile tobacco decoction (boiled)	1	0
<i>Experiment III</i>			
Reynolds' Natural Leaf (1), chewed, hands wet with saliva and this rubbed on plants when set	3	0
<i>Experiment IV</i>			
Kate Gravely, plug	3	0
Spark Plug, do	3	0
Masterpiece, do	3	0
Buster, do	3	0
Penn's Pen., do	3	0
Maritiana, do	3	0
Tinsley's Natural Leaf, do	3	0
Boot Jack, do	3	0
Check	3	0

TABLE 2 (Continued)

Brands	No. plants inoculated	No. plants mosaic	Incubation periods in days
<i>Experiment V</i>			
Days Work, plug	5	0
Schnapp's, do	5	0
Fish-hook (1), do	5	0
Climax (2), do	5	0
Brown's Mule (2), do	5	0
Star, do	5	0
Apple Suncured (2), do	5	0
Composite of Old Loyalty, Five Bros., Bull Durham, Stud, Duke's Mixture and Granger (rough cut), Granulated Smoking	5	2	8
<i>Experiment VI</i>			
Composite of Tiger, 8-hour Union, Pay Car, Beechnut and Mail Pouch	5	0
Bruton's Scotch Snuff	5	0
Dark tobacco 5-years old, not commercial	5	5	7, 7, 7, 9, 11
Chesterfield cigarettes (1)	5	1	11
Fresh mosaic material	3	3	5
<i>Experiment VII</i>			
Climax (1), chewed and saliva rubbed on hand and plants pulled and set	3	3	21, 25, 25
Checks, (clean hands)	3	0
<i>Experiment VIII</i>			
Lucky Strike (1), cigarette	3	2	11, 15
Camel (1), do	3	1	11
Chesterfield (2), do	3	3	8, 10, 10
Reynolds' Nat. Leaf (2), plug	3	1	14
Apple Suncured (3), do	3	2	10, 14
Brown's Mule (3), do	3	0
Drummond (2), do	3	0
Reynolds' Nat. Leaf (3), do	3	0
Brown's Mule (4), do	3	0
Drummond (3), do	3	0
Apple Suncured (4), do	3	2	9, 16
Reynolds' Nat. Leaf (4), do	3	2	16, 35
Drummond (4), do	3	0
Brown's Mule (5), do	3	1	19
Apple Suncured (5), do	3	1	9
Climax (3), do	3	0
Checks (rubbed with juice from healthy plants)	3	0

TABLE 2 (Continued)

Brand ^a	No. plants inoculated	No. plants mosaic	Incubation periods in days
<i>Experiment IX</i>			
Old Loyalty, gran. smoking.....	3	3	9, 13, 17
Five Bros., do	3	3	13, 14, 15
Stud, do	3	3	13, 14, 15
Bull Durham, do	3	3	9, 13, 14
Duke's Mixture, do	3	3	12, 14, 17
Granger Rough Cut, plug cut.....	3	0
Check (rubbed with one of own leaves)	3	0
<i>Experiment X</i>			
Fish-hook (1), plug.....	3	0
Mosaic tobacco steamed 105° C. 10 min.	3	0
<i>Tomato Plants</i>			
Chesterfield (2), cigarette.....	3	2	25, 25 (suspicious in 9 days)
Reynolds' Natural Leaf (1), plug	3	†	Appeared very mild in 3 plants
Climax (1), do	3	†	Appeared very mild in 3 plants
Camel cigarettes (1).....	3	1	25 (suspicious in 9 days)
<i>Experiment XI</i>			
Chesterfield cigarettes (3).....	3	3
Check	1	0
<i>Experiment XII^d</i>			
Chesterfield (4), cigarette.....	5	2	16, 17
Camels (2), do	5	0
Lucky Strike (2), do	5	3	8, 9, 9
Herbert Tareyton, do	5	2	9, 9
Piedmont, do	5	3	12, 14, 18
Fatima, do	5	5	5, 9, 13, 14, 14
Melachrino, do	5	5	9, 9, 9, 13, 14
Checks	14	0

^a The number following the brand indicates the particular sample of this brand used.

^b Plants inoculated with a concentrated decoction, the decoction being injected into several parts of the plants with a hypodermic needle. Plants which remained healthy in the previous test used again with the same brand of tobacco.

^c The plants of the above series which remained healthy were again inoculated with a second decoction of their respective plugs and again remained healthy.

^d A separate cigarette, swab, etc., was used for each of the above plants. Two check plants were used for each brand of cigarettes, one being inoculated with leaves from two and the other with leaves from three of the five plants to be inoculated. This is a check on the method of inoculation and also demonstrates that the plants used were free from mosaic when the experiment was begun.

failed to obtain infection, except in one plant, when manufactured twists have been used as the source of inoculum. One manufacturer informed us that it had been found necessary to steam the twists before packing for shipment to prevent spoiling.

Before storing in hogsheads, Burley tobacco is run through a redrier at temperatures ranging from 140° F. to 170° F. for 40 minutes. This has no apparent effect on the virus, as 18 plants inoculated with tobacco redried at 150° F., 18 with tobacco redried at 165° F., and 18 with tobacco not redried, all gave 100 per cent infection, while the checks remained healthy. The samples used were selected at random at the redrying plant and represent the results which may be expected from cured Burley leaf in general. Burley tobacco, therefore, when purchased by the manufacturer, is viruliferous and, if it goes through no processes which destroy the virus during manufacture, should be so in the final product. The same is probably true of other types of tobacco.

A number of brands of commercial tobacco have been tested in the course of these studies to see to what extent they may carry the virus. Preliminary inoculations were made with plug tobaccos which gave positive results in some cases, but the results are open to question as the plugs were not flamed and may therefore have carried small particles of infected tobacco, as dust, into the decoction. Later inoculations, recorded in table 2, were made with greater care; tools were sterile, and the plugs of tobacco were always carefully flamed. A portion of the plug was then shaved off onto a clean piece of letter paper and poured from this into an Erlenmeyer flask containing a small amount of sterile water. The inoculations were made by the finger-rubbing method, the hands being washed with soap and running water between inoculations. The person making the inoculations touched nothing but the soap, water, plants, and decoction during any set of inoculations, another person shifting the pots, etc., and pouring the decoction on the operator's hands. In all cases except the last lot of cigarette inoculations, the checks were inoculated last by rubbing the leaves of healthy plants with the wet fingers after washing.

The results seem to indicate definitely that occasional plugs of tobacco carry the mosaic virus, and possibly that certain brands are likely to be found to be rather consistent carriers of the disease, although others may carry it only rarely or possibly be free. Much more extensive work is of course necessary to determine these points. However, four of five plugs of Apple Suncured, three of four plugs of Reynolds' Natural Leaf, two of five plugs of Brown's Mule, and one of three plugs of Climax tested were found to give positive results; whereas of four plugs of Drummond tested, all gave negative results. Nineteen other plugs of miscellaneous brands gave only negative results.

The shredded chewing tobaccos to which sweetening is added appeared to be free from the virus and probably are put through a sterilizing process in manufacture. The granulated smoking tobaccos and cigarettes tested appear to be viruliferous, as positive results were obtained from all of them. Chesterfield cigarettes were used in a plant pathology student laboratory exercise and as a result of carefully controlled inoculations three plants became infected.

Of a total of 45 commercial brands of tobacco tested, infections were obtained from the following 16 brands:

Plug	Granulated Smoking	Cigarettes
Reynolds' Natural Leaf	Old Loyalty	Chesterfield
Apple Suncured	Five Brothers	Lucky Strike
Brown's Mule	Stud	Camel
Climax	Bull Durham	Herbert Tareyton
	Duke's Mixture	Piedmont
		Fatima
		Melachrino

SUMMARY

The results reported indicate that, in regions where cured tobacco is commonly chewed by tobacco growers, it may be an important source of mosaic infection, especially at weeding and pulling time. Commercial chewing tobaccos probably are not so important a source of mosaic infection, as far as tobacco is concerned, as the natural leaf, the results, except for four brands of plugs, being negative. Cigarettes and granulated smoking tobaccos also probably play a very minor part in the mosaic problem in commercial planting of tobacco. They are likely to be of importance, however, in connection with more limited plantings of susceptible plants such as tomatoes grown under glass or intensive culture in the field. Under these conditions the plants are handled from time to time in setting, pruning, training, and harvesting. Infection of a single plant under such conditions may lead to the spread of the disease through the house or field. Although it has not yet been conclusively demonstrated, it might be expected that the fingers of an inveterate cigarette smoker would become contaminated with the mosaic virus and thus transfer it to plants which were handled. Cigarettes are also an important consideration for the investigator who smokes cigarettes and works with susceptible plants. It is not at all improbable that many cases of sporadic mosaic infection occurring in cultures of tomatoes and tobacco in mosaic-free houses may have originated from fingers which have become contaminated while smoking cigarettes.

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THE EFFECT OF A STRAIN OF TOBACCO MOSAIC ON THE YIELD AND QUALITY OF BURLEY TOBACCO¹

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In a test carried on the past season to determine the effect of mosaic on quality and yield of Burley tobacco, such striking results were obtained that they are being published, although they cover but a single season, in the hope that they may stimulate further investigation. Growers in Kentucky pay very little attention to mosaic when it appears in the fields in the usual amounts of a trace to 10 or 12 per cent after setting, taking it to be an abnormal condition of the plant beyond their control. Occasionally, when most of the plants are affected, it causes considerable concern. If the plants grow fairly normally, many growers believe the cured tobacco is not injured, as no sign of the mosaic pattern remains after curing. The question as to whether later infections, such as those occurring at topping or suckering stages, cause injury to the cured tobacco has hardly been given consideration. In this connection, Chapman (p. 81)² states: "The amount of damage done by late mild attacks when the plants are maturing, or appearing on the sucker growth after topping, is practically negligible and, so far as can be learned, does not in any way injure the commercial leaf."

Two distinct types of mosaic are commonly found in tobacco fields in Kentucky. One of these causes severe stunting, distortion and, in the field, burning of the leaves, together with a distinct mottling; the other type causes only a slight retardation of growth, the leaves are rarely distorted or rugose, and the mosaic pattern is not conspicuous. The first causes marked reduction in yield and quality if inoculations are made soon after setting. The other, the mild type, does not cause marked injury. The mild type of mosaic was used in the following experiment:

Thirteen rows of standup White Burley tobacco (strain 36-12) were set with a setter, June 9, 1926. The odd rows were left as buffer rows in an attempt to prevent, as much as possible, the spread of mosaic from row to row. The second and eighth rows were inoculated at setting time, or as soon as growth commenced if the first attempt was not successful. The fourth and tenth rows were inoculated at topping time, and the sixth and twelfth rows were not inoculated. The disease spread some during the summer, but care was used in selecting plants for final harvest, in the rows

¹ Published with the approval of the Director of the Kentucky Experiment Station.

² CHAPMAN, G. H., *Mosaic diseases of tobacco*. Mass. Agr. Exp. Sta. Bul. 175. 1917.

TABLE 1.—Effect of mild mosaic on the yield and quality of Burley tobacco at Lexington, Kentucky, in 1926

Grade	Healthy uninoculated			Inoculated at topping			Inoculated at setting		
	Actual yield, 50 plants, gms.	Per cent	Advance per 1,000 lbs.	Actual yield, 50 plants, gms.	Per cent	Advance per 1,000 lbs.	Actual yield, 50 plants, gms.	Per cent	Advance per 1,000 lbs.
Flyings	533	13	\$ 9.02	180	4	\$ 3.06	429	16	\$10.83
Second trash	773	19	24.30	561	14	17.71			
do				279	7	6.09			
do				313	8	3.80	755	27	13.61
Lugs	598	14	14.46	515	12	12.50			
do							333	12	7.21
Leaf	727	18	15.83	340	8	7.43			
Leaf, green cast				830	20	4.03			
Red	1,250	30	21.16						
do				703	17	6.83	983	35	14.18
Tips heavy	254	6	2.46	398	10	3.86	273	10	3.94
Total	4,135	100	\$87.23	4,119	100	\$65.31	2,773	100	\$49.77
Per cent decrease in crop-value due to mosaic		0			25.1			43.1	
									\$61.7

* Assuming an acre yield of 1,000 pounds for the "healthy" and for the "inoculated at topping" plants, the yield per acre of the "inoculated at setting" plants would be 671 pounds.

inoculated at topping time, which appeared healthy up to the time of topping (August 11, 1926). The hands were washed in soap and water before topping the healthy rows. As a further precaution, these plants were examined nine days before cutting and only the plants with mosaic-free suckers were suckered, thus marking them for harvest. All plants in the other rows were suckered at the same time. Fifty plants were harvested from each of the three pairs of rows twenty-eight days after topping. The tobacco was all cured together, was stripped and sorted into hands, and was then taken to the Burley Cooperative Association warehouse where it was graded by the head grader of the Association and his assistant. The grade, advance paid by the Association, the actual yield in grams of each grade, the percentage of the total yield represented by each grade, and the advance for each grade on an acre basis, figuring the yield at 1,000 pounds per acre, are given in table 1.

The yield was reduced 33 per cent by infection at setting time, although not appreciably reduced by infection at topping time. The leaves of plants inoculated at setting time averaged about three or four inches shorter than the leaves of the other two lots, and were of lower quality. The leaves of plants inoculated at topping time fell into nine grades; whereas those from healthy plants fell into only six grades. This is an important factor, as it increases the difficulty of grading or, if the grading is not carefully done, reduces the value of the better grades because of admixtures of lower grades. The effect of mosaic seems to be evident even in the lower leaves (second trash and flyings) but is probably most marked in the quality of the leaves which expanded following inoculation.

In the leaves which expanded (grades D to F) there was a marked effect on color, 20.15 per cent of the crop falling into DMI which is "leaf" with a green cast. The E grade or red was greatly reduced in weight, and was of a lower grade than in the healthy plants, a portion of it evidently having fallen into the DMI grade. It seems certain that mosaic infection, even though not occurring until topping time, has a marked effect on the color of cured tobacco, causing it to be dark or to have a green cast.

The advance paid for the various grades of tobacco by the cooperative association, although not an accurate measure of its total value, may be used for comparison. A reduction of 33 per cent in yield due to inoculation at setting time resulted in a reduction of 43.1 per cent in value of a given weight of tobacco and 61.7 per cent reduction in value on an acre basis. Inoculation at topping time, although resulting in no appreciable reduction in yield, caused a reduction of 25.1 per cent in the value of the crop. This difference could hardly have been predicted from the appearance of the plants at cutting time. The result emphasizes the necessity of developing

cultural practices which will not only eliminate initial mosaic infection but will reduce the spread of the disease throughout the entire growth period of the plants.

It has been recognized for years that late inoculation, even in the upper portion of the plant, would result in the entire plant becoming viruliferous in a comparatively short time. That the presence of the virus in well-developed, apparently normal leaves has any effect upon them either while alive or during the curing process has not been generally recognized even by tobacco specialists, it being commonly believed that the chief loss caused by mosaic is due to stunting of the plant and to loss of quality in the mottled leaves.

Miss Eckerson³ gives a possible basis for an understanding of the results reported in the present paper. After inoculation of a single leaf on a tomato plant, she found small flagellate organisms penetrating the chloroplasts of other leaves and partially destroying them, noting that later some cells were completely disorganized. If similar effects are found to be produced in mature tobacco leaves, it might be expected that during the curing process many of the partially disorganized chloroplasts, which have not lost their color completely, might not break down normally, but remain as they were, thus accounting for a darker color in some leaves and the distinctly green cast in others. The partial or complete destruction of other cell constituents following inoculation may also have some effect on the color of the cured product.

SUMMARY

1. Two types of mosaic occur commonly in the tobacco fields of Kentucky. One type produces severe stunting accompanied by distortion and a distinct mottling, together with a "burning" of the leaves; the other type causes only a slight retardation of growth, without an apparent quantitative or qualitative injury.

2. A study was made of the effect of the mild type of mosaic on the yield and quality of Burley tobacco, grown in the field in 1926.

3. There was no appreciable reduction in yield when the plants were inoculated at topping time; but infection at setting time resulted in a one-third yield reduction as compared with the check.

4. The leaves of plants inoculated at setting time averaged about three to four inches shorter and were of lower quality than the leaves of either the check or those inoculated at topping time, resulting in a reduction of 43.1 per cent in value of a given weight of tobacco and 61.7 per cent reduction on an acre basis.

³ Eckerson, Sophia H. On organism of tomato mosaic. Bot. Gaz. 81: 204-209. 1926.

5. The leaves of plants inoculated at topping time fell into nine grades; whereas those from healthy plants fell into only six grades, thereby increasing the difficulty of grading.

6. Inoculation at topping time, although not reducing the yield, nevertheless resulted in a reduction of 25.1 per cent in the value of the crop, which difference could hardly have been predicted from the appearance of the plants at cutting time.

7. Possible disorganization of chloroplasts during the curing process, and the partial or complete destruction of other cell constituents following inoculations, may be responsible for the color of the cured product.

8. There is a real necessity for developing cultural practices which will not only prevent initial mosaic infection, but will reduce the spread of the disease throughout the entire growth period of the plants.

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THE BLACK WALNUT (*JUGLANS NIGRA* L.) AS A CAUSE OF THE DEATH OF APPLE TREES¹

F. J. SCHNEIDERHAN

IN 1923 the writer observed an instance of incompatibility between a black walnut and apple trees in an orchard near Winchester, Virginia. The walnut, with a limb spread of about 40 feet, had apparently dwarfed two apple trees and killed six others in a circle around it. Digging up the roots of the injured apple trees revealed the fact that they were intermingled with walnut roots about 45 feet from the trunk of the walnut. This was the first instance that came to the writer's attention of injury resulting from close association of these two species of trees. In 1926, when a definite effort to find additional cases of this antagonistic relationship was made, we were informed by a farm hand employed for years on an old Virginia estate that he had been aware 30 years ago of the injurious effect of walnuts on apple trees. According to his version, it was rather generally known at that time that walnut trees injured or killed apple trees planted nearby.

The antagonism of certain species of *Juglans* to other plants is not a new observation. The notes of A. H. Gilbert reported by Jones and Morse² in Vermont indicate that the shrubby cinquefoil (*Potentilla fruticosa* L.) was killed in areas about the base of butternut trees (*Juglans cinerea* L.). This area extended beyond the limb spread of the butternut. Examination of the roots of the cinquefoil showed that whenever they came in contact with, or were in close proximity to, the butternut roots, they were killed. The death of the weed in this instance was always associated with close contact with the butternut roots. Where an outcropping of rock prevented the growth of the butternut roots, the cinquefoil grew normally.

Another instance of walnut antagonism to other species was noted by Mel. T. Cook,³ who reported the wilting of tomato and potato plants growing in the immediate vicinity of the black walnut. He writes:

"Attention has been called from time to time to a number of cases of wilting of potato and tomato plants which was undoubtedly due to walnut (*Juglans nigra*) trees growing in the immediate vicinity. The plants show a decided wilting but do not lose

¹ Paper No. 71, from the department of botany and plant pathology, of the Virginia Agricultural Experiment Station.

² JONES, L. R., and W. J. MORSE. The shrubby cinquefoil as a weed. Ann. Rept. Vt. Agr. Exp. Sta. 16: 188-190. 1902-1903.

³ COOK, MEL. T. Wilting caused by walnut trees. Phytopath. 11: 346. 1921.

their color or die, as in the case of plants that have been attacked by wilt-producing fungi or bacteria, or struck by lightning. The range of the wilting coincides very closely with the spread of the root system."

In an unpublished note, Fromme describes this type of injury to tomato plants as follows:

"On several occasions in Virginia, a wilting of tomato plants growing in proximity to black walnut (*Juglans nigra*) has been noted. The first observation was made near Amsterdam in 1916. Areas of wilted plants were seen in two separate fields of tomatoes, and each area centered on a walnut tree growing in the fence row. The rapid wilting of the plants suggested bacterial wilt (*B. solanacearum*), but examination failed to reveal this organism. On inquiry it was learned that the occurrence of this type of wilt in proximity to walnut trees was a matter of common observation among farmers in the locality."

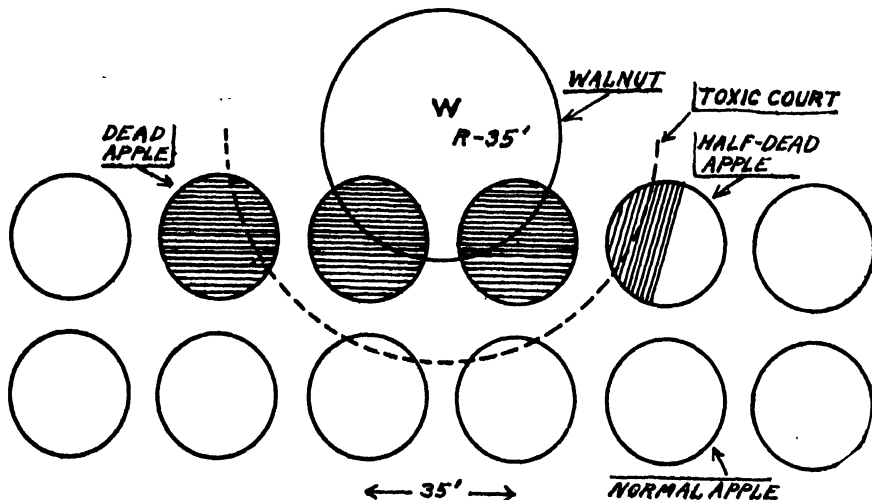


FIG. 1.—Walnut with a limb spread of 70 feet and a toxic court with a radius of 51 feet has caused the death of three York apple trees and the dwarfing of a fourth.

A more detailed study of the antagonism of walnut to other plants, together with the toxic constituent of the walnut, was made by Massey⁴. His conclusions are as follows, based on a study of the injury to alfalfa, tomatoes and potatoes:

- "1.—Walnuts (*Juglans nigra* and *J. cinerea*) have an antagonistic action which causes a wilting and dying of certain plants such as alfalfa, tomato and potato.
- "2.—Roots of the affected plant were always in close contact with walnut roots; the toxic substance is not generally distributed in the soil around the walnut trees, but localized in the vicinity of the walnut roots.

⁴ MASSEY, A. B. Antagonism of the walnut (*Juglans nigra* L., and *J. cinerea* L.) in certain plant associations. *Phytopath.* 15: 773-784. 1925.

“3.—Walnut root bark contains a substance which is toxic to the roots of tomato plants grown in water culture.

“4.—It is likely that juglone, or some similar substance, is the toxic constituent of the walnut.”

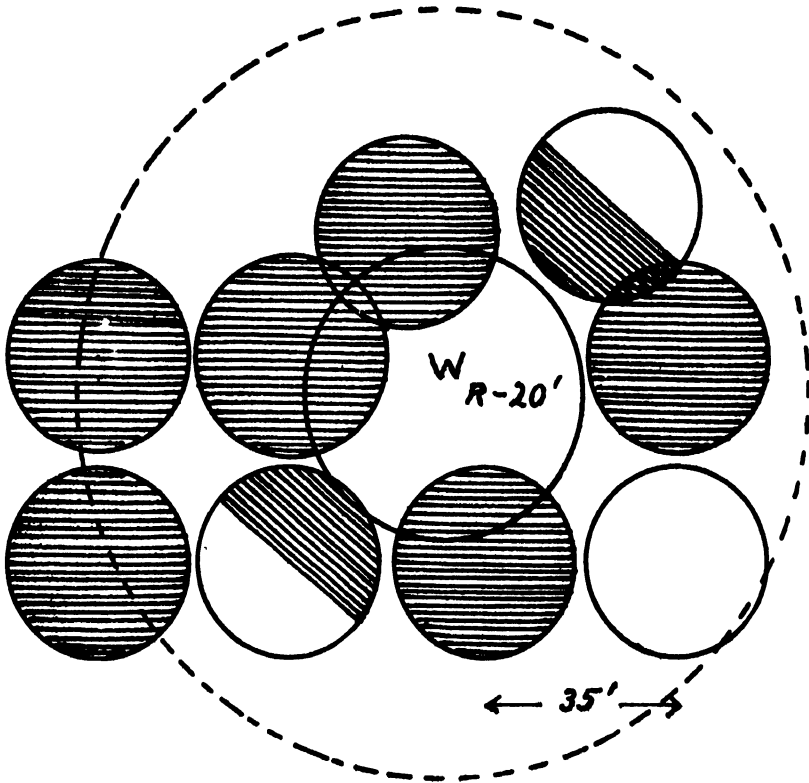


FIG. 2.—Walnut tree with a limb spread of 40 feet and a toxic court of 66 feet has caused the death of six York and Pippin trees and the dwarfing of two.

No evidence has been presented, so far as the writer knows, that species of *Juglans*, other than *J. nigra* and *J. cinerea*, are toxic to other plants, particularly the apple. There is, in fact, considerable evidence to the contrary with respect to species in common use as stocks for the Persian or English walnut. In a letter of November 2, 1926, to F. D. Fromme, Robert W. Hodgson, of the University of California, writes with reference to the injury in question:

“In my ten years of experience with the walnut in California, where we now have more than 100,000 acres, I have never seen any results of this kind. To be sure, our trees are not propagated on the eastern black walnut, *Juglans nigra*, but are either on the Persian seedling stock or one of the other of the two California black walnuts. Fully 50 per cent of our walnut acreage is intercropped, and practically all types of field and truck crops have been used successfully with no apparent evidence of injury. Thousands

of acres of tomatoes and potatoes are grown in young walnut orchards in Southern California. It is not at all uncommon to find apple trees in walnut orchards, and apparently doing very well."

The two California black walnuts referred to are *Juglans hindsii*, the northern black walnut of California, and *J. californica*, the southern black walnut, as explained by Professor Hodgson in a letter of later date. He states that *J. hindsii* is most commonly used as a stock.

The writer has observed the non-injurious effect of the Persian or English walnut (*J. regia*) on apples on the grounds of the Winchester Field Laboratory. A row of these walnuts was planted about ten years ago next to a row of Stayman apple trees. The distance between these trees is 27 feet. The Stayman trees are growing normally in spite of the fact that the

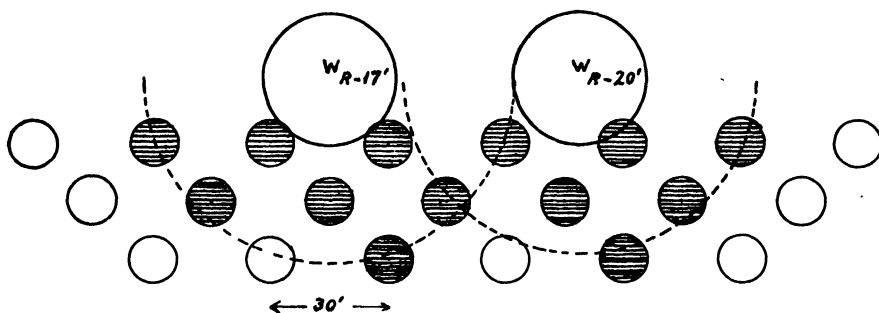


FIG. 3.—Two walnuts with limb spreads of 34 and 40 feet and toxic courts with radii of 46 and 45 feet, respectively, causing the death of 13 York trees.

roots and branches intermingle with the walnut. In addition, the roots of these walnut trees extend through a plat of ground in which Japanese barberry, spirea, English ivy, phlox, tulips, petunias, iris, castor bean, roses, and other flowers and shrubs have been planted. No injury of any kind has been observed during the past four years as a result of this association.

Our search for additional instances of walnut toxicity to apple trees was made in July and August, 1926. This work was incidental to our regular fruit disease investigations at Winchester. Only two days were given entirely to search for walnut injury to apples. In these two days we found seven instances along the country highways, and undoubtedly numerous additional cases could be found. Our purpose was merely to discover, if possible, additional cases of this type of injury without studying specific toxic products of the walnuts as causative factors. We found it to be a comparatively easy matter to discover instances of walnut injury while the foliage was still on the trees, a missing tree or an injured one being very noticeable at that time. The various measurements recorded later in this paper were made in January, 1927.⁵

⁵ The writer is indebted to Dr. F. D. Fromme and Dr. S. A. Wingard, of the Virginia Experiment Station, for assistance in making measurements and for suggestions regarding the preparation of the diagrams. Acknowledgment of this assistance is hereby made.

The apple plantings in Frederick County, Virginia, are probably as extensive per unit area as in any part of the United States. The black walnut (*J. nigra*) is one of the commonest nut-producing trees in Virginia, and the wood of this tree has been used extensively in furniture manufacture since colonial days and is still used extensively in factories specializing entirely in walnut furniture. Thus we were able to find 18 instances of walnut injury, 13 of which were selected for description in this paper.

Judging by the ease with which we found 18 instances of walnut injury, we are of the opinion that the aggregate loss of apple trees caused by walnut toxicity is considerable in this state.

From the diagrammatic representation of the apple trees killed or injured by walnuts in the following pages, together with the photographs, the reader will be able to see that, with few exceptions, apple trees were either

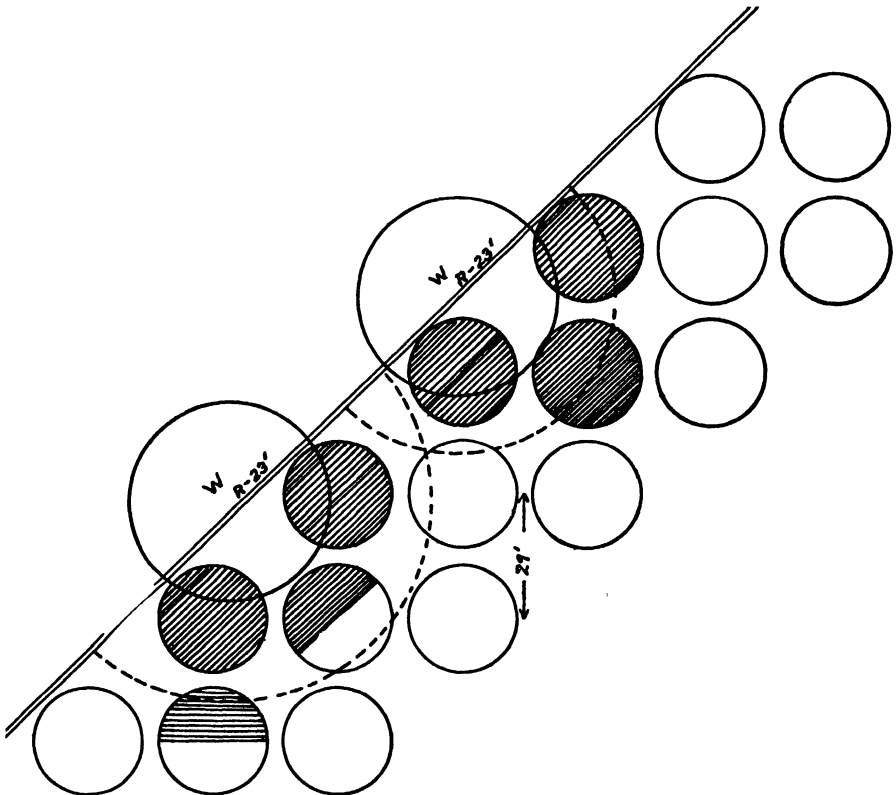


FIG. 4.—Two walnuts with limb spreads of 46 feet each and toxic courts with radii of 38 and 48 feet, causing the death of five York trees and the dwarfing of one tree inside and another outside of the assumed limits of the toxic courts.

killed completely or partly, or dwarfed when growing within the approximate limits of root spread—the toxic court surrounding the walnut. Usually this court is represented as a sector with an arc of 180 degrees because most of the instances of injury were found along fence lines bordering the orchards. In only one instance (Fig. 2) did we find a walnut growing within an orchard. Digging of roots to determine their extent and direction was not resorted to except in the case of the walnut tree in figure 2. In this instance we found that the walnut and apple roots intermingled, with unquestionable injury to the latter. The work of Massey indicates that there is considerable variability in the direction and extent of walnut root growth. The so-called “death line” of alfalfa plants surrounding a walnut indicates that the roots of the walnut extended from 33 to 64 feet from the trunk. Whenever uninjured apple trees were found within the limits of the toxic court, we assumed that the roots of the walnut had not developed in that direction. The fact that the average depth of soil in Frederick County (Virginia) is very shallow and that outcroppings of limestone shelves are frequent and numerous lends plausibility to this assumption. Of course there may be causes, other than walnut toxicity, of the injury and death of some of the apple trees described in this paper. Such causes may be mouse injury, shallow soil, root rot fungi, and other diseases. However, the close association of dead or injured apple trees with walnut trees, and

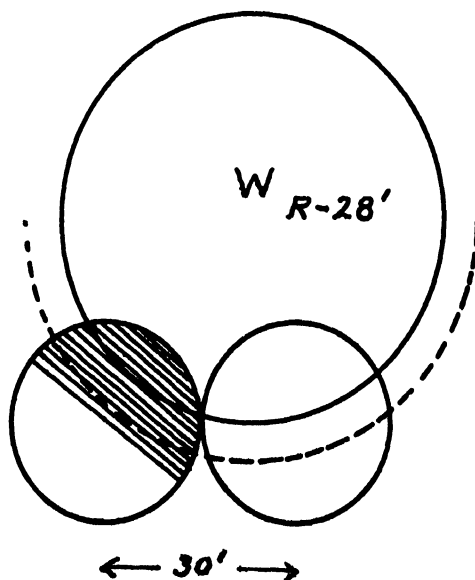


FIG. 5.—Walnut with limb spread of 56 feet and a toxic court with a radius of 36 feet, causing severe dwarfing of a Ben Davis and no apparent injury to a Stayman tree within the toxic court.

the regularity of this occurrence, lead us to conclude that walnut toxicity is the outstanding cause of death of apple trees in these specific cases.

An interesting observation requiring further study was made in regard to the comparatively small extent of walnut injury to trees of the Stayman variety. It appears that this variety either escaped injury in three instances or else possesses a varietal resistance to walnut toxicity not found in such varieties as the York, Ben Davis, and Pippin. Notable among these instances is the one represented in figure 5. In this instance, we found a Stayman tree growing normally at a distance of only 25 feet from a very large walnut; whereas a Ben Davis tree standing only 8 feet farther away was not only severely dwarfed, but all of the limbs on the side toward the walnut were dead. In another instance, we discovered two large Stayman trees uninjured at a distance of 43 feet from a large walnut, although in the same orchard row about six rods distant three Ben Davis trees were killed by a walnut growing at approximately the same distance.

These observations of the behavior of Stayman are too limited to make definite deductions. There is a possibility, nevertheless, that resistance to walnut toxicity by Stayman may be demonstrated. The explanation that presents itself is that this variety may have developed scion roots which may be resistant to the toxic constituent of the walnut roots. It is quite apparent that the ordinary crab stock on which our common varieties are grafted is not resistant.

The writer has used diagrams in describing five of the thirteen instances of walnut toxicity. The symbols used in the diagrams (Figs. 1-5) are explained here.

The largest circles with the letter W in them denote walnut trees. The letter R in these same circles followed by a figure indicates the radius, in feet, of the limb spread of the walnut. The fully shaded smaller circles denote dead apple trees. The partly shaded circles indicate seriously injured or dwarfed apple trees. The dotted lines, usually in the form of a semi-circumference, denote the approximate extent of the toxic court. In figure 1 these symbols are identified by a printed legend.

DISCUSSION OF FIGURES 1 TO 5

Figure 1. Typical injury of apples resulting from close proximity to a large walnut tree. The large limb spread of the walnut in this instance was 70 feet, and the approximate extent of the toxic court, as represented in the diagram, comprises a sector with a radius of 51 feet. Irregularity of walnut root development is indicated in this diagram, as the dwarfed tree is at exactly the same distance from the walnut as the dead trees. Figure 6 is a photograph of this instance of walnut toxicity. This protograph also shows



FIG. 6.—The toxic court of the walnut described in figure 1. Note the two small replant in the foreground, one of which is indicated by the flag.



FIG. 7.—Two large walnuts which caused the death of 13 apple trees. The toxic courts of these walnuts are shown diagrammatically in figure 3. Some of the places occupied by the dead trees are marked by flags.

two small replants in the place of the dead trees. It is obvious that these replants will also be killed unless the walnut tree is removed.

Figure 2. This represents the first instance of walnut antagonism to apple trees noted in this study. After pointing out the injurious effect of this walnut to the owner of the orchard, the walnut was removed. This instance of incompatibility is noteworthy because it represents the largest number of apple trees killed by one walnut. Furthermore, we have here a comparatively small walnut with an extensive root system. The extent of the toxic court has been represented by a circle with a radius of 60 feet. The varieties are York and Pippin.

Figure 3. The loss of 13 trees was caused by two walnuts. In this instance, however, there were filler trees between the regular rows, which increased the number of tree deaths. Figure 7 shows a photograph which amplifies the diagram in figure 3.

Figure 4. Typical toxic courts are shown in a York orchard. The walnut trees in this instance are large, but the number of dead apple trees is comparatively smaller than in some other instances where walnut trees are smaller. Figure 8 is a photograph of this case of toxicity.

Figure 5. A very large walnut has severely dwarfed one side of a Ben Davis tree growing at a distance of 38 feet. A Stayman, on the contrary, growing at a distance of only 30 feet, was not injured. This is the smallest toxic court observed around so large a walnut tree.

EIGHT ADDITIONAL CASES OF WALNUT TOXICITY

No. 1.—In this instance a walnut with a limb spread of 50 feet and surrounded by a toxic court with a radius of 48 feet caused the death of two York trees within the toxic court and severely dwarfed another tree six feet outside of it. Two other trees outside the toxic court were also killed. To assign walnut toxicity as the cause of the death of the two last mentioned trees is questionable. However, the irregularity of root growth of walnut trees may account for this, but other factors may be involved. If the trees outside of the toxic court were killed by the walnut tree, they represent the maximum distance at which apple trees have been killed in this observation. The distance in this case was 80 feet.

No. 2.—In a well managed Ben Davis orchard the only missing trees were found to be within the toxic court of a walnut tree with a limb spread of 52 feet, the toxic court having a radius of 43 feet. As these are the only trees missing in the entire orchard and are found next to a walnut tree, there is little doubt that the walnut tree caused the death of the apple trees.

No. 3.—In the same orchard shown in figure 7, a walnut tree with a limb spread of 46 feet and a toxic court of 43 feet caused the death of three York



FIG. 8.—The large walnuts at the right of the picture caused this toxic court in which 5 trees were killed and 2 severely injured on the side toward the walnut trees. This instance of walnut toxicity is described diagrammatically in figure 4.

trees within the toxic court and the severe dwarfing of one tree standing 8 feet outside of the limit of the court.

No. 4.—A walnut tree with a limb spread of 48 feet and a toxic court of 36 feet caused severe dwarfing of two Ben Davis trees within the limit of the toxic court and of another tree seven feet outside of this limit. The dwarfing in all three instances was on the side of the tree toward the walnut.

No. 5.—Five Ben Davis trees were dead and a sixth severely dwarfed near a small walnut tree with a limb spread of only 46 feet and a toxic court with a radius of 66 feet. The dwarfed tree was outside of the toxic court.

No. 6.—A small walnut tree with a limb spread of 34 feet and a toxic court of 54 feet caused the death of three trees within the toxic court and the dwarfing of another tree outside of it. In this instance, four trees standing within the toxic court were uninjured.

No. 7.—A Stayman tree growing at a distance of 25 feet from a walnut was uninjured, whereas a Black Twig standing 43 feet from the walnut was dead. No other dead trees were found in this orchard. The limb spread of the walnut was 38 feet.



FIG. 9.—Walnut injury in one side of a Ben Davis tree. All of the branches on the side toward the walnut tree in the background have been killed. Figure 5 explains the toxic court of this walnut.

No. 8.—A large walnut with a limb spread of 52 feet apparently caused no injury to two Stayman trees 43 feet away. These Stayman trees were in the same row as the Ben Davis trees described as case No. 2. In view of the fact that the walnut tree is approximately of the same size and growing at the same distance from the Staymans as the walnut near the Ben Davis trees, it would seem to indicate varietal resistance of the Staymans to walnut toxicity.

SUMMARY

The data presented in the thirteen instances of walnut toxicity indicate that a total of 16 walnut trees have apparently caused the death of 48 apple trees of different varieties, or an average of 3 apple trees per walnut. A total of 14 apple trees were either dwarfed or injured on one side by these same walnuts; the average distance of the dead trees from the walnuts is 39 feet; the average distance of the dwarfed and partly injured trees is 47 feet. The maximum distance at which an apple tree was probably killed by walnut toxicity is 80 feet. The average limit of the toxic court is a sector drawn with a radius of 50 feet from the walnut as a center. The maximum

extent of this toxic court is such a sector with a radius of 70 feet. The average limb spread of walnuts in this study was 46 feet, and the circumference of the walnut trunks, 3 feet from the ground, was 5 feet and 4 inches. The ratio of the trunk circumference to the limb spread in feet is as 1 to 8.4. The average limb spread of apples examined in this survey is 13 feet. There is no correlation between the extent of walnut limb spread and the toxic court. This is shown very strikingly by the fact that one walnut tree with a limb spread of 56 feet has a toxic court of approximately 36 feet; whereas another walnut tree, with a limb spread of only 34 feet, has a toxic court of approximately 66 feet.

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VARIETAL SUSCEPTIBILITY OF ADA RED AND CERTAIN OTHER APPLE VARIETIES TO CEDAR RUST, WITH SPECIAL REFERENCE TO TWIG INFECTIONS

V. H. YOUNG

Varietal differences in the susceptibility of certain kinds of apples to leaf and fruit infection with cedar rust have been commonly noted, and certain writers have further observed that some varieties are also susceptible to twig infection. Reed and Crabill (4) find that on the variety Smith's



FIG. 1.—Cedar rust lesions on apple nursery stock of the variety Ada Red. $\times 1\frac{1}{2}$.

Cider the twigs are frequently seriously diseased by cedar rust. Giddings (1) pictures mature aecia of the cedar rust pathogen on wild crab apple twigs and, more recently, Hopkins (2) reports marked susceptibility to twig infection in the Yellow Bellflower. Jones and Bartholomew (3) also report twig infection on the wild crab in Wisconsin, and Weimer (4) reports twig infection of apples with cedar rust. So far as the writer knows, the relation of twig infection to young nursery stock has not been considered.

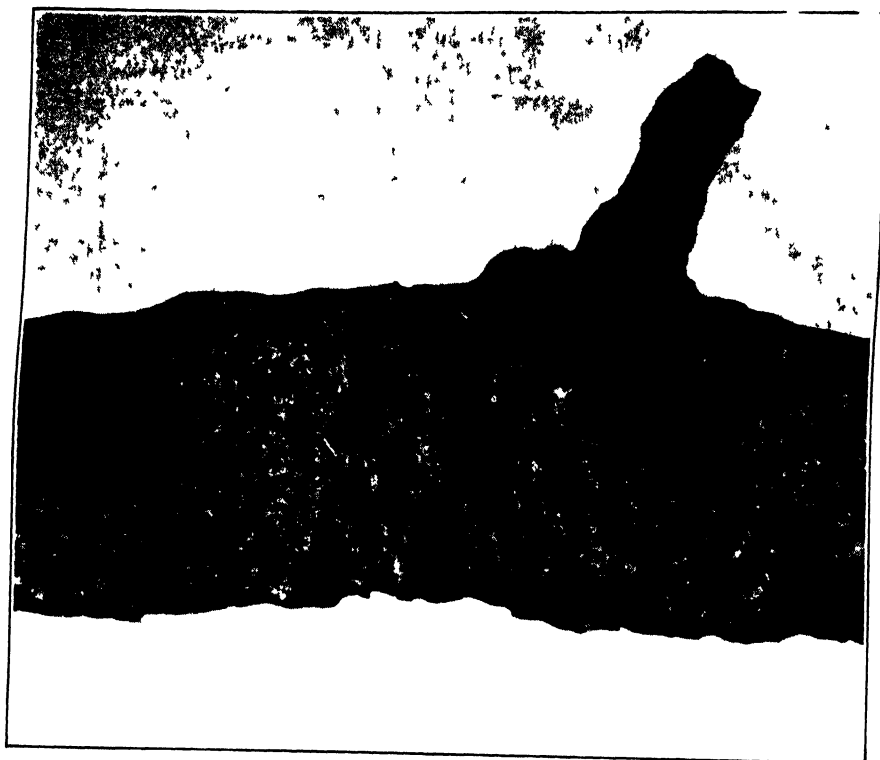


FIG. 2.—Mature aecia on cedar rust lesion on apple nursery stock. $\times 4$.

During the fall of 1926, Mr Sam E. Poe, of the staff of the Arkansas State Plant Board, called the writer's attention to a serious attack of cedar rust on the wood of one-year-old whips of the variety Ada Red. In one nursery in Benton County, Arkansas, in a block of several thousand one-year whips of this variety, more than 9 per cent of the trees were badly affected with cedar rust on the main stem. In another nursery, a few miles away, about 7 per cent of the trees in a block of the same variety were affected. Blocks of other standard varieties such as Delicious, Winesap, and Arkansas Black in the same nurseries were not affected with twig infec-

tions. In practically all cases the injury occurred about a foot above the crown and was in the form of a deep-seated canker, which in many cases had completely girdled the stem (Fig. 1). Many of the trees were so severely affected that they broke off when slightly bent, and early in the fall a large number of them broke at the point of infection from the weight of a light snowfall. Such trees in many cases were found to be dead above the point of infection, and in all cases the injury was so severe as to render the trees worthless. No shortening and thickening of the growth, such as is noted by Weimer (5) in twig infections, was seen.

Mature aecia were noted by the writer on many of the cankers, and upon microscopic examination the peridial cells and the contained spores were found to agree closely in their measurements and appearance with those of *Gy. nosporangium juniperi-virginianae* Schw. (Fig. 2). Twig infection on mature trees of the variety Ada Red has been commonly noted in northwest Arkansas by the writer and by others, but apparently little serious injury results in most cases. This variety, in common with the Jonathan, Golden Delicious, and certain other varieties, often suffers severely from cedar rust infections on the leaves, and to a less extent from infections on the fruit. Infection of the wood of one-year whips of Ada Red is apparently a much more serious matter than twig infection of older trees, and results in a total loss of the year's growth. As was noted by Hopkins (2), wood infections appear to originate near buds rather than on the internodes, and from the position of most of the cankers a few inches from the crown it is inferred that only the apical portion of a growing twig is susceptible to infection.

Severe infection on the wood of nursery stock has been noted in one case on the Golden Delicious, and twig infections on older trees are fairly common in northwest Arkansas on the varieties Jonathan, Ada Red, Benoni, Duchess, and Golden Delicious. It is worthy of note that the leaves of all these varieties, with the possible exception of Benoni, are also extremely susceptible to cedar rust.

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FAYETTEVILLE, ARKANSAS.

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DOTHICHIZA POPULEA AND ITS MODE OF INFECTION

GEORGE G. HEDGCOCK

Dothichiza populea Sacc. and Briard causes a serious canker disease of some species of *Populus*, especially in the Northern and Northeastern United States and Southeastern Canada (2, 3, 4). This fungus, apparently introduced from Europe where it was first described in 1884 [according to Delacroix (1)], attacks most severely the Lombardy and Norway poplars (*Populus nigra italica* and *P. eugenei*).

The life history of *Dothichiza populea* is not fully known. The writer (3), during his earlier studies of this disease, observed that cankers are almost invariably formed at the nodes, and frequently around the bases of immature dead twigs. Detmers (2), in 1923, stated as follows, regarding its mode of infection: "Infection takes place at the nodes. The discolored area may be immediately below the leaf scar, in the axil of the bud, or on either side of the bud in proximity to the stipular scars. The bud itself is not attacked. Cankers form at any node on two-year-old wood." Her observations as to the location of the infection the second year are correct, but convey the impression that the disease gains entrance through the leaf and stipular scars, and axils of the buds.

The writer in 1922 carried out two experiments to ascertain the manner of infection. Young healthy trees of *Populus* and *Salix* without bark lesions or cankers were grown in a bed near the greenhouses of the U. S. Department of Agriculture at Washington, D. C. In one experiment the following trees were inoculated: 3 *Populus alba* L., 10 *P. alba* L. var. *pyramidalis* Bunge., 3 *P. deltoides* Marsh., 5 *P. eugenei* Simon-Louis, 7 *P. nigra* L. var. *italica* Du Roi, 10 *Populus* sp., and 3 *Salix* sp. To obtain spores for inoculation, the lower ends of twigs of *Populus nigra italica* infected by *Dothichiza populea* were placed in water under a bell jar until the spore tendrils exuded from the pycnidia. On May 13, 1922, the tendrils were removed and dissolved in sterile tap water and the spore suspension was applied with a sprinkling can to the leaves, twigs, and limbs of the trees mentioned above.

By August 1, many of the inoculated leaves on the species of *Populus* that later became cankered had become discolored with gray to black or brown irregular leaf spots, which gradually increased in size. The dead areas later extended to the petioles and darkened them. By September 15 some of the leaves and petioles were killed, and by October 15 the discolora-

tion had progressively spread from the leaf blades to the petioles and from them to a number of the smaller greener and more succulent twigs, which gradually blackened, shriveled, and died. The following spring, small discolored areas or cankers appeared in the bark of the trunks at the bases of the dead twigs, and in three instances at the bases of dead leaves at the nodes. Twig infections were noted on the following trees: 5 *Populus nigra italica*, 1 *P. deltoides*, 3 *P. eugenei*, and 4 *Populus* sp. All the infected trees of these species bore infected leaves. Cankers with fruiting tendrils of *Dothichiza populea* appeared at the bases of diseased twigs in the spring of 1923 on 5 *Populus nigra italica*, 2 *P. eugenei*, and 2 *Populus* sp. Control trees, one of each species, remained free from infection.

Dothichiza populea was reisolated from the tissues of the freshly diseased leaves, petioles, twigs, and cankers previously described, after sterilizing their surfaces with a solution of mercuric chloride in distilled water, 1 to 1,000. The fungus was obtained from 5 out of 6 leaf cultures, 5 out of 6 petiole cultures, and 6 out of 6 twig cultures.

On May 12, 1922, a number of other trees were inoculated by inserting spore horns obtained as in the other experiment in wounds made in the bark by means of a sterile scalpel. After inoculation the wounds were completely covered with a wrapping of dry, sterile absorbent cotton. The following trees were inoculated: 4 *Populus alba*, 4 *P. alba pyramidalis*, 4 *P. deltoides*, 4 *P. eugenei*, 7 *P. nigra italica* and 4 *Salix* sp. For controls, one tree of each species was similarly treated, but no spores were inserted in the wounds. None of the controls became infected. Of the inoculated trees, 3 *Populus nigra italica* and 1 *P. eugenei* were infected, and by September 15 small cankers appeared around the wounds. The cankers developed rapidly and produced the spores of the fungus by April, 1923. The tops of all four trees had also died above the cankers.

These experiments, though rather crude and carried out in the open under natural conditions, are offered as evidence that *Dothichiza populea* may infect poplar trees by progressive infection from the leaves through the petioles to the twigs and limbs, and also by direct entrance through wounds. They do not disprove, however, that the fungus may enter also through leaf and stipule scars and bud axils. Under natural conditions this fungus produces a large crop of viable spores in the spring about the time the poplar leaves are expanding. Repeated attempts by the writer to secure cultures of the fungus from infected specimens collected in autumn indicate that it produces few if any spores at that time. For this reason infections probably do not occur in late autumn after leaf scars have formed. Infections probably occur at the time the spores are produced, or soon thereafter.

It is possible that the entrance of the fungus through the leaves in springtime might be prevented, especially in nurseries, by spraying the trees with bordeaux or other fungicides about the time the leaves are expanding, and again after each heavy rain for at least one month thereafter. The writer has had no opportunity to test the effect of spraying and offers the suggestion to others who may have an opportunity to do so.

Publication of the results given here has been delayed in the vain hope that the experiments might be repeated with more refined technique, but they are now offered for what they may be worth to other investigators of the subject.

Since the first account of the occurrence of the disease caused by *Dothichiza populea* in the United States (3), the known range of the fungus has been greatly extended. Much of this extension has very probably resulted from the shipment of diseased nursery stock. The fungus has been collected from Maine southward to Virginia and westward to Minnesota, Nebraska, and New Mexico, on the following species of *Populus*: *P. brevifolia*, *P. deltoides*, *P. deltoides canadensis*, *P. eugenei*, *P. nigra italica*, *P. petrowskiana*, *P. simonis*, and *P. simonis fastigiata*.

SUMMARY

Experiments carried out under natural conditions indicate that *Dothichiza populea* Sacc. and Briard may infect poplar trees by progressive infection from the leaves through the petioles to the twigs and limbs, and also by direct entrance through wounds.

Spraying with bordeaux is suggested as a probable means of preventing the fungus from entering through the leaves in the springtime.

Since the first report of the disease in North America, the known range of this fungus has been greatly extended in Northern and Northeastern United States and Southeastern Canada, probably through shipment of diseased nursery stock.

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THE NEMATODE DISEASE OF SWEET POTATOES¹

R. F. POOLE AND ROBERT SCHMIDT

It has been known for some time that the sweet potato, *Ipomoea batatas*, is susceptible to the eel worm disease, *Heterodera radiculicola* (Greef) Muller (1, 2), but only recently has definite information been published regarding the susceptibility and resistance of sweet potato varieties (5). Nematode root knot is a major disease of many crops throughout the South and in greenhouses in many parts of the North. Its importance is further emphasized because the worm not only injures the root system, resulting in malformed roots and stunted plants, but it is accused of opening the tissues of roots and stems so that they become susceptible to fungi that cause the severe root rot and wilt diseases.

The 1926 season was an extremely favorable one for nematode infestation of plants in North Carolina. Cotton, soybeans, cowpeas, okra, beets, parsley, tomatoes, tobacco, carrots, lettuce, and celery are a few of the crops that were badly diseased in the field. The severity of the infestation should warrant an immediate presentation of any information on varietal resistance.

PLAN OF EXPERIMENT

The experiment had its origin as a varietal test for adaptability and quality of sweet potatoes conducted at the Coastal Plain Station, Willard, North Carolina, by the Horticultural Department. The infested soil at the station is of the sandy nature that is characteristic of the Atlantic coast from New Jersey to Florida. The test plots were sown in winter oats which were cut and plowed under about May 1. The soil was then prepared for sweet potatoes. A high grade fertilizer was applied in the ridge at the rate of 600 pounds an acre. On June 8, one week later, the plants were set out 12 inches apart in 3.5-foot rows. The following varieties were used: Red Jersey, Yellow Jersey, Big Stem Jersey, Porto Rico, Triumph, Southern Queen, Norton Yam, Nancy Hall, Yellow Yam, Red Bermuda, and Pumpkin Yam. Related strains of these varieties used were Vineless Yellow Jersey, Improved Big Stem Jersey, Old Long Red, Early Carolina, General Grant, Myers Early, Dixie Yam, Miles Yam, White Yam, and New Gem.

¹ Published with the approval of the Director of the North Carolina Experiment Station as paper Number 19 of the Journal Series.

NATURE OF NEMATODE INFESTATION

On some varieties, the stems, roots, and potatoes were infected. Knots were very prevalent on the small roots (Plate XIX, A), where the sizes varied from that of a pin point to three millimeters in diameter. The knots on the small potatoes (Plate XIX, B) were also large and protruded prominently. In only a few large potatoes out of thousands examined were the knots well developed and raised above the surface of the potato skin (Plate XIX, C, and Plate XX, A), for in most instances the roots were badly malformed, with deeply indented, rough scabs (Plate XIX, C and D). Even the larger potatoes were affected severely, there being uniform infection over

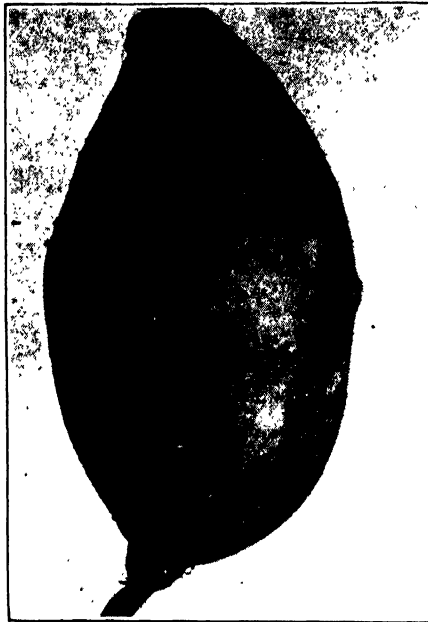


FIG. 1. Prominent scab-like infected areas on Yellow Jersey variety.

the entire surface (Plate XX, C). The indenting of circular areas around the potato roots (Plate XX, A) was a constant symptom on the severely infected varieties, where newly developed sprouts had been attacked and destroyed. The female worms were abundant in the knots on roots and on small and large potatoes (Plate XX, B, D) being most numerous along the margins of the large physiological cracks, both on small and large potatoes. They apparently were first attracted to these broken tissues that could be entered more readily than healthy parts. In some cases the points of entrance into the potato centered around the new sprouts and the roots that developed on the sides of the potatoes. Infection was most severe in

the cracked or broken tissues. The females contained an abundance of eggs, some of them with nearly mature worms. The males were not abundant at harvest. The few infected areas on the Jersey varieties (Fig. 1) were distinctly scab-like ones in which the cortical cells were broken and split open.

RELATION OF VARIETIES TO INFECTION

When examined early in October, the vines on severely infected varieties were not well developed. At that time a decision was made to obtain data on infection at harvest, which was October 21. It so happened that the varieties were alternated and repeated in such a satisfactory way that uniform infestation of the soil on the test plot was made certain.

Some relations of varieties to infection, yield under infected conditions, and remarks are given in table 1. Of the Jersey and Porto Rico varieties

TABLE 1.—*The relative susceptibility of important commercial sweet potatoes to nematode disease*

Variety	Infection (per cent)	Yield (bu. per acre)	Remarks
Red Jersey	0.8	245.0	No infection of roots.
Yellow Jersey	1.0	228.0	Very slight infection, confined mostly to ends of small roots.
Big Stem Jersey	1.2	238.0	Very slight infection, confined mostly to ends of small roots.
Porto Rico	1.2	237.0	No infection of roots, but slight infection of cracked potatoes only.
Triumph	5.8	120.5	Very slight infection of roots.
Southern Queen	35.5	107.3	Uniform but slight infection of roots.
Norton Yam	85.0	71.1	All roots badly diseased.
Nancy Hall	100.0	81.7	All roots and potatoes severely diseased, roots badly broken—potatoes severely marked.
Yellow Yam	100.0	28.1	All roots and potatoes severely diseased, roots badly decayed and broken—potatoes severely marked.
Red Bermuda	100.0	26.6	All roots and potatoes severely diseased, roots broken.
Pumpkin Yam	100.0	32.5	All roots and potatoes severely diseased, roots broken.

there was only an occasional knot on the roots and a few blister-like areas on the potatoes. The infection on these varieties was so insignificant as to be of no economic importance. On the roots of Triumph plants there were many knots, but only slight infection of potatoes. The roots of the Southern Queen showed minor infection, but the potatoes were irregularly, although severely, marked. Weimer and Harter (5) found no infection on this variety in tests made in California. They also reported no infection on the Red Jersey, which was only slightly attacked in these tests. Otherwise the results given in this paper are in agreement with those they obtained. Infection was very severe on Norton Yam, Nancy Hall, Yellow Yam, Red Bermuda, and Pumpkin Yam. The potatoes were so severely marked that it was impossible to make accurate counts of recently infected areas (Plate XX, C). The New Gem and Dixie Yam, which are strains closely related to the Porto Rico variety were only slightly infected. There was only slight infection also of the Jersey varietal strains, Improved Big Stem Jersey, Old Long Red, and Early Carolina; the varieties General Grant and Myers Early, which are related to Nancy Hall, were severely diseased. White Yam and Miles Yam, which are closely related to Southern Queen, had slightly infected roots and severely infected potatoes.

The severely infected varieties showed a marked reduction in yield compared with that of the more resistant varieties. The actual loss due to nematodes is suggested in the yields, since the Nancy Hall, Southern Queen and Yellow Yam normally yield as well as the Porto Rico. In most well fertilized soils the Southern Queen will yield better than the Porto Rico and Jersey varieties. The Porto Rico yield of 153.3 bushels an acre more than the Nancy Hall is largely owing to nematode infection of the latter. On the other hand, the Pumpkin Yam and the Red Bermuda may yield poorly on any soil, and their low yield in this test may be partly due to other factors, as it is well known that some of the varieties do not yield well on all soils (3). However, it may be safely stated that the low yields in these tests were due in great part to nematode infection.

DISCUSSION

It is probably most interesting to note here that varieties both susceptible and field-immune to wilt-producing species of *Fusarium*, were attacked by nematodes. The Nancy Hall variety is not only very susceptible to nematodes but is one of the most susceptible to wilt or stem rot. The Yellow Yam, which is immune from wilt or stem rot, was severely attacked by nematodes. The only wilt resistant variety that showed any worth while resistance to the nematode disease was the Triumph, but it is not a market favorite and would not be grown extensively for that reason. This brings

about an extremely complex situation in which the wilt- or stem-rot resistant varieties can not be used safely on soils infested with nematodes and with the causal *Fusaria* of wilt. Where such infections occur it will be interesting to revert to the skill of cultural methods, since they have been used successfully in reducing losses and maintaining normal yields.

Close planting and the use of two or three plants in hills formerly set with one has not only maintained a normal stand but produced a satisfactory yield (4). This is still the real hope of fighting the stem-rot disease on most infested soils, and should receive further attention in the South where the nematodes are likely to cause severe losses of the favorite varieties: Nancy Hall, Southern Queen, Yellow Yam, and Norton or Dooley Yam.

Porto Rico, the favorite variety in the South, is very susceptible to stem rot, and since it normally produces many "jumbos" (a term applied to potatoes larger than the market grades), could be set as close as 8 to 12 inches without reducing the yield of marketable potatoes. Under most conditions this could be counted on to maintain a normal stand and give a satisfactory yield on severely infested soils. The close plantings would certainly prevent the formation of large potatoes, and aid somewhat in satisfying market demands.

The Yellow Jersey varieties are very susceptible to stem rot or wilt but resistant to nematodes. Furthermore, the big commercial plantings of these varieties are north of the areas where the nematode is of economic importance in the field.

SUMMARY

The nematode or eel worm disease of sweet potatoes is the cause of heavy losses in the eastern part of North Carolina, especially on sandy soils. Cotton, soybeans, cowpeas, okra, beets, parsley, tomatoes, tobacco, carrots, lettuce, and celery are a few of the crops that were badly diseased in the field in 1926.

The popular commercial and other strains of sweet potato varieties which were tested on the infested soils showed wide variation in resistance and susceptibility. The Porto Rico and Jersey varieties were very resistant to the nematode, while all other important varieties, Norton Yam, Yellow Yam, Southern Queen, Red Bermuda, and Nancy Hall, were very susceptible.

Root infection of resistant varieties was very slight, there being only a few definite blister to scab-like scars on the potato. On the susceptible varieties the symptoms were so-called knots and scabs on stems, roots, and potatoes. On the susceptible varieties malformation was produced; scabby areas, followed by pit-like rots, were prominent; but raised pustule-like areas were not very abundant on potatoes.

Infestation was very prominent on potatoes that were cracked sometime during the season by physiological changes. The female worms were abundant in these areas and were full of eggs, which contained nearly mature worms.

Varieties susceptible to *Fusarium* wilts are resistant to the nematode, and vice versa. For instance, it would not be safe to use the Southern Queen, Yellow Yam, and Red Bermuda varieties, which are resistant to stem rot, in nematode-infested soils. Where both parasites are likely to be abundant, it is suggested that the Porto Rico and Jersey variety strains be planted, because of their resistance to nematodes. For the control of stem rot a sufficiently large number of plants should be planted per acre to insure a satisfactory stand after the period of heavy infection. This is done by setting plants 8 to 12 inches apart in the row, or 2 to 3 plants in 18- to 24-inch hills.

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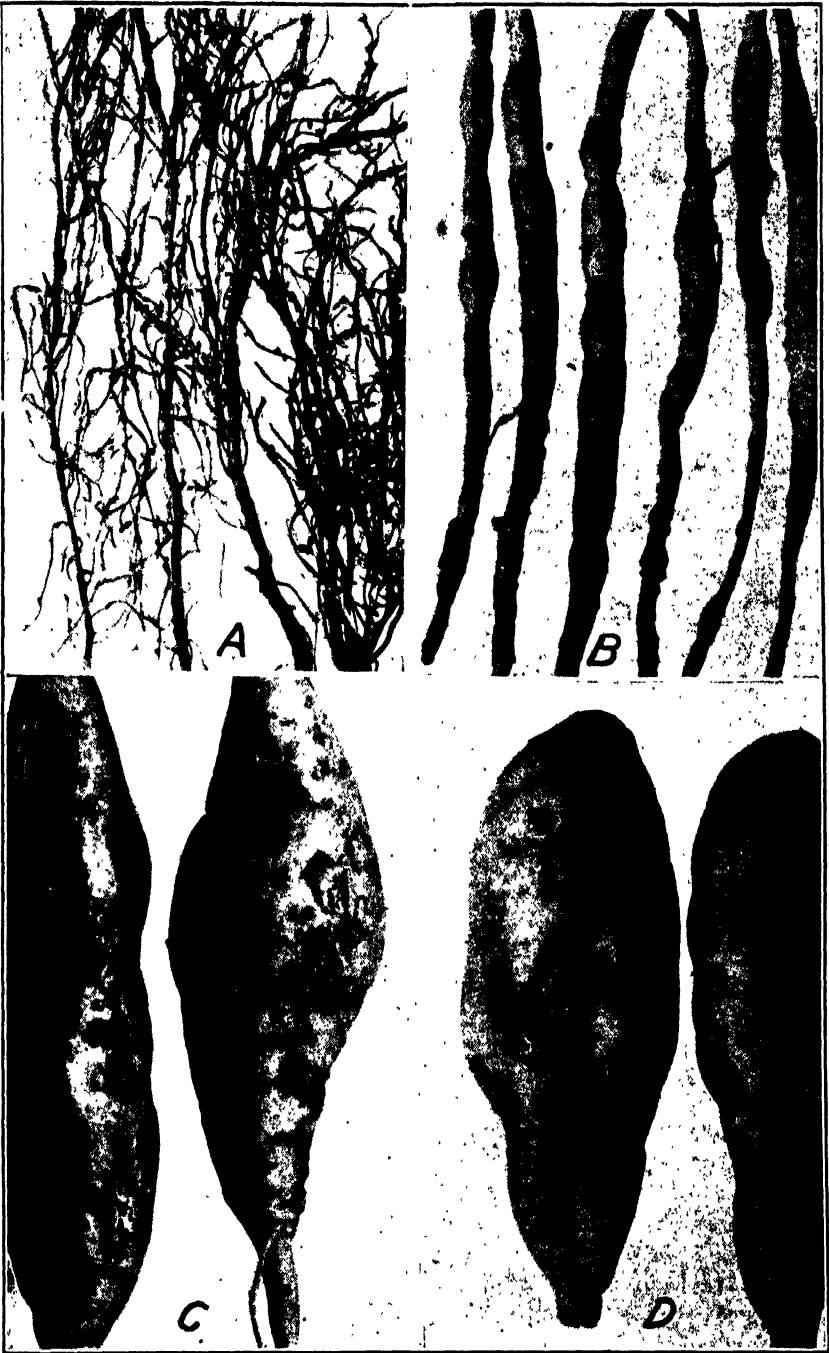
DESCRIPTION OF PLATES

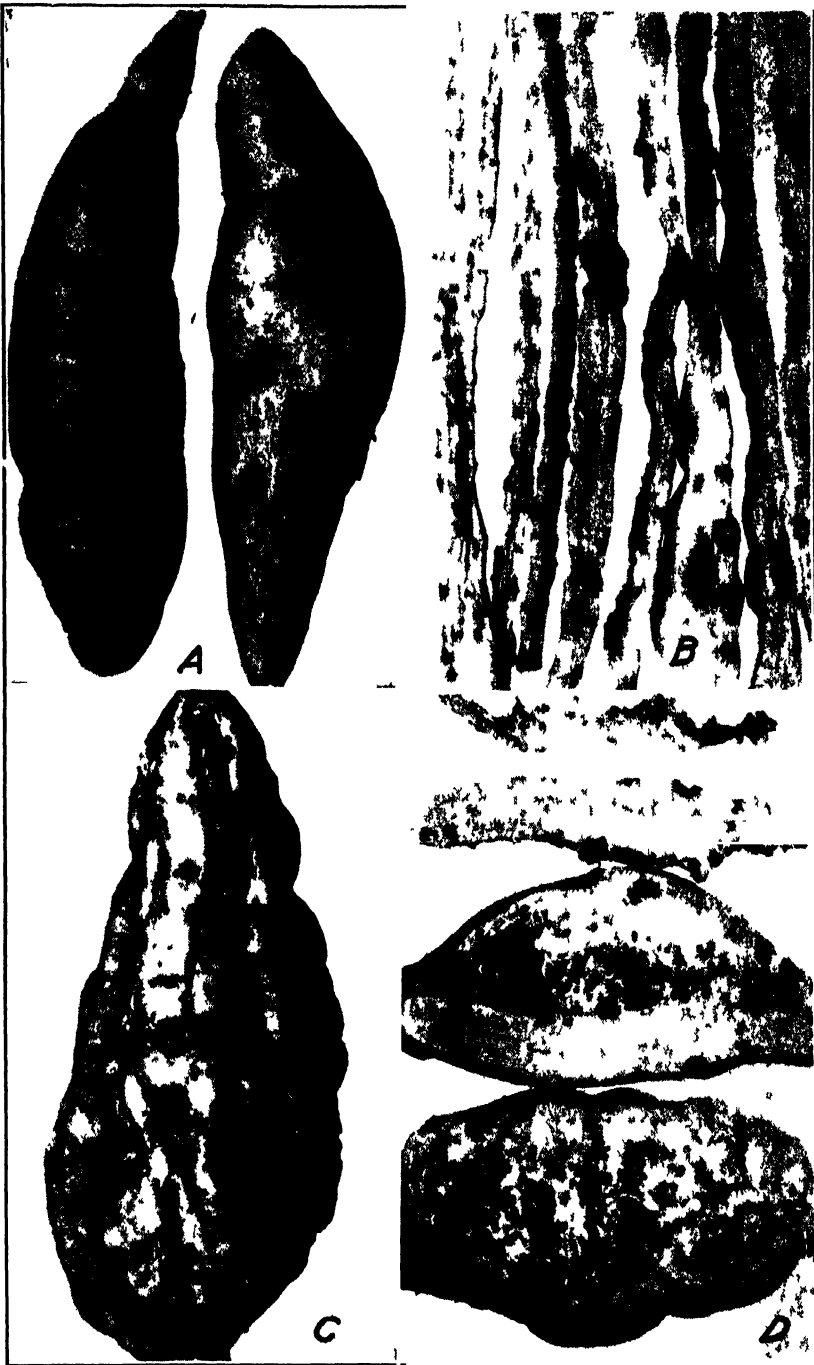
PLATE XIX

- A. Malformations or knots on small roots of Nancy Hall sweet potato.
- B. Small potatoes badly diseased, and malformed.
- C. Coarse scabby formation on surface; most severe on potatoes physiologically cracked.
- D. Scab and pit areas on sprouting points.

PLATE XX

- A. Scab areas extending around the potato.
- B. Small potatoes and roots, in which female nematodes were abundant.
- C. Large potato severely attacked by worms.
- D. Large potatoes, in which female nematodes were numerous; greatest infection on physiologically cracked areas.





NET NECROSIS OF THE POTATO

A. H. GILBERT

Some confusion appears to exist in the literature in regard to the terminology of potato tuber necroses. This is recently illustrated in an article entitled *Net Necrosis of the Potato* by D. Atanasoff (1). Atanasoff, in this article seems to have arrived at quite different conclusions from those reported in the United States by the present writer and others, and appears to have taken the term "net necrosis" from its connotation here and applied it to certain tuber and vine symptoms occurring in Holland, which symptoms, as described, are quite distinct from those associated with net necrosis in this country.

There are evidently several types of necrosis in potato tubers, both in Europe and in America. These include tuber discolorations variously known as brown fleck, internal brown spot, internal rust spot, sprain, streak, and net necrosis. The divergent use of these terms by various investigators, and others, indicates that there is a considerable lack of uniformity in their application. It appears, however, that there are some features more or less common to a group of these diseases and on the basis of which they may be distinguished from net necrosis. First, they affect various internal areas of the tuber but not specifically the phloem of the vascular tissues; second, the necrotic conditions are not progressive during storage as in the case of net necrosis; and last, these necroses are not transmitted by the tuber with the result that there are any characteristic observable effects upon the plants grown from affected tubers.

In noting the several necrotic conditions that may be observed in potato tubers, it should not be forgotten that frost necrosis, as described by Jones, Miller and Bailey (11), produces a browning and blackening of the vascular region and phloem strands which, in at least one of the several types, closely resembles net necrosis.

The writer has recently undertaken a study of net necrosis, of the phloem-necrosis type, as it occurs in tubers of the Green Mountain variety, and although the investigation is still under way and quite incomplete, it has seemed justifiable to make some preliminary statements in connection with general comments on the paper by Atanasoff (1).

In the first place, the point to be emphasized in opposition to the view of Atanasoff is that the net necrosis first noted and described by Orton (5) is the net necrosis which has more recently been extensively investigated by

Schultz and Folsom (8, 9, 10) and found to be associated intimately with leafroll. Results secured by the writer (2), including observations covering a period of several years, fully agree with those of Schultz and Folsom (8, p. 78), who state that "net necrosis is apparently a leafroll symptom, being a discoloration which results from tuber phloem necrosis and which appears more often as conditions of variety, recency of infection, and weight of tuber are more favorable. It develops in the dormant tubers without relation to differences in the storage temperature."

Kasai (3), reports from Japan a similar condition of tuber necrosis which was correlated with leafroll. Positive results in the way of transmission were secured by him by means of tuber grafts and also with juice inoculations. In a certain lot of leafroll tubers used for grafting, he reported flesh discolorations in twelve tubers. Kasai (3, p. 52), writes as follows in regard to the necrosis he described: "This, in all probability, is identical with the net necrosis of the tubers described by Schultz and Folsom (1921)."

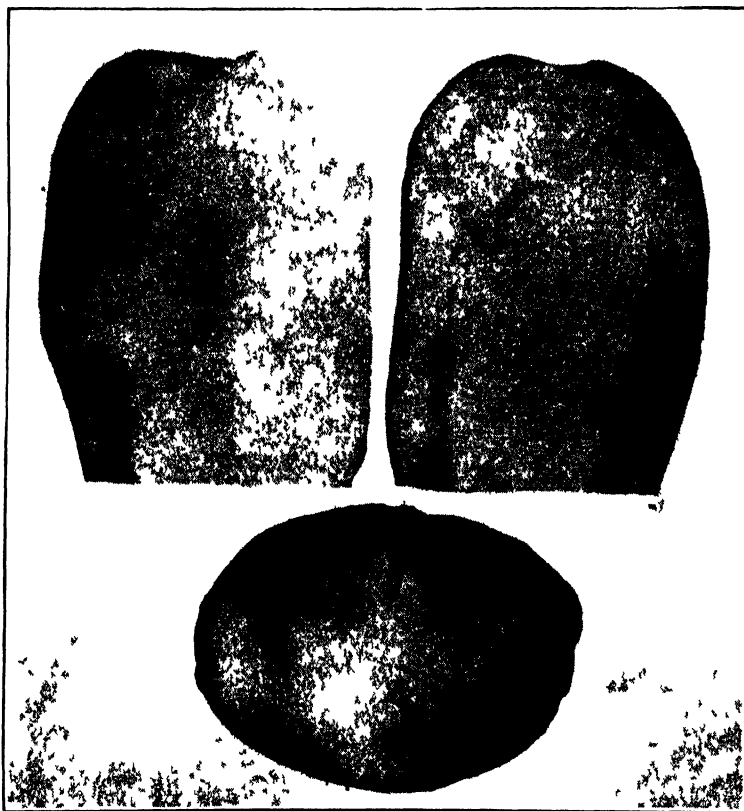


FIG. 1. Cross and longitudinal sections of a Green Mountain tuber with well marked net necrosis.

Net necrosis of the tuber as the writer has studied it may be described as a degeneration, browning, and ultimate dying of the cells of the phloem strands and phloem parenchyma, both outside and inside the vascular ring of the tuber. This degeneration and browning begins at the stem end and progressively affects the tissues, above mentioned, with varying severity toward the bud end (Fig. 1). The necrotic areas, so far as conspicuous browning is concerned, are not always continuous from the stem end on, but often discontinuous. In addition to the browned phloem strands of the vascular ring and of the outer and inner phloem regions, there are also frequently areas of irregular outline and of a water-soaked appearance in these regions, generally centering in the main vascular ring. The necrosis of the phloem seldom extends entirely to the bud end of the tuber.

The significance of the term "net necrosis" as descriptive of this trouble may be noted if the skin and outer cortex of the affected tuber are removed, when the branching and anastomosing strands of the outer phloem appear as a conspicuous brown network (Fig. 2).

In 1926 a favorable opportunity presented itself for studying the relation of net necrosis to leafroll, when an unusual outbreak of the disease occurred in the stock of a large producer in Vermont. In the grading of certain lots for spring shipment, 10 or 15 per cent of necrotic tubers were found. Several bushels of these tubers were secured, and selected necrotic tubers were planted in tuber units in an experimental plot. Other rows were planted

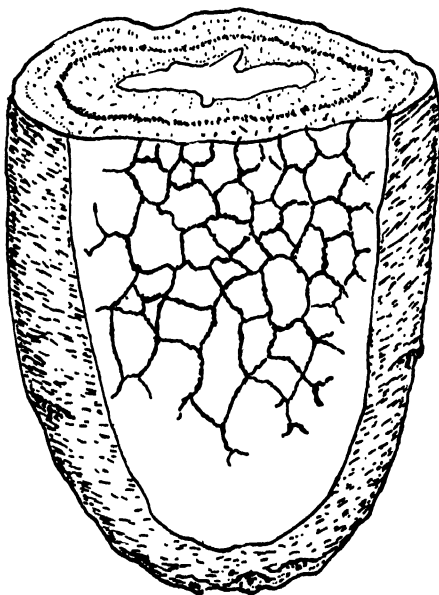


FIG. 2. Diagrammatic view of portion of necrotic tuber. The removal of epidermis and outer cortex reveals the brown, anastomosing strands of the necrotic phloem.

in tuber units with tubers as they came in the sacks, some necrotic and some free from necrosis. Still other rows contained units of healthy stock. An excellent opportunity was thus afforded to observe the progeny of these necrotic tubers in comparison with other stocks.

The necrotic tubers in tuber units produced practically 100 per cent leafroll hills (Fig. 3). In a total of two hundred hills there was not a single healthy unit and only a few hills where the leafroll symptoms were not clear. The rows planted with tubers as they came in the sacks, that is, without sorting out the necrotic ones, contained 25 per cent leafroll plants. This



FIG. 3. Experimental field at Burlington, Vt. The three rows at the right were planted with necrotic tubers in tuber units. There are many weak, and some missing, hills and practically 100 per cent leafroll units.

result, following similar results obtained in the two previous seasons, offers convincing evidence that, in the Green Mountain variety, there is an intimate relationship between the necrosis and leafroll.

Schultz and Folsom (8) mention "recency of infection" as one of the conditions associated with the appearance of net necrosis. This means that

necrosis is generally limited to tubers produced by plants the same season in which they have become infected with leafroll. According to Quanjer's definition (7), this is "primary" infection. Conspicuous leafroll symptoms may be present, or they may be entirely absent.

The observations of the present writer strongly indicate that net necrosis is a first-season symptom of leafroll following primary infection. In confirmation of this, many tubers from leafroll plants have been examined, both in the fall and at the close of the storage period, but well marked cases of necrosis have not been found. On the other hand, as stated above, necrotic tubers have almost invariably produced leafroll plants. This situation may explain why Atanasoff failed to find net necrosis upon the examination of several hundred leafroll tubers. In accordance with the above-mentioned observations, this was the result to be expected.

An examination of the illustrations of necrosis in Atanasoff's article would suggest that he might be picturing two distinct types of necrosis. The symptoms shown in his figure 2, in the variety Ashleaf, could hardly by any possibility be net necrosis of the phloem-necrosis type, as we understand it in America. In his illustration the necrotic areas do not follow the vascular ring, but are, in some of the slices, distinctly in the pith region, a condition which does not exist in phloem necrosis.

The tuber discolorations in the variety Roode Star, as shown in figure 3 of Atanasoff's paper, somewhat resemble net necrosis, but the subsequent history of the plants grown from such tubers would indicate that some other necrotic condition was present. The facts that the necrotic symptoms in the variety Roode Star were often confined to the stem end and that they were mistaken for *Phytophthora* lesions point strongly to the probability that the necrosis in these cases is distinct from the net necrosis as it occurs in the United States and as defined in the present paper.

Again, the correlation between the Holland necrosis of the tubers and aucuba mosaic of the vines makes it quite certain that the writer is dealing with a set of symptoms widely at variance with those associated with net necrosis in this country.

The occurrence of spindling sprout in connection with necrosis and leafroll has been observed repeatedly both by Schultz and Folsom (8, 9, 10) and by the writer (2). It is not stated that spindling sprout is a constant symptom of leafroll, but that it is of frequent occurrence in this connection, and, further, that it is quite consistently associated with net necrosis. It is often observed that some of the sprouts produced by a tuber in late storage are spindling, while others are normal. It will be noted in such cases that the spindling sprouts are toward the stem end, while the sprouts at the bud end may be normal. This does not mean the production of a healthy plant

from the terminal eyes, for the tuber acts as a unit in such cases in the production of leafroll plants. The plant or plants from the terminal buds will often be more vigorous than those from the eyes toward the basal end but will exhibit at least mild leafroll symptoms (Fig. 4). Further investigation will generally reveal that the part of the tuber producing the thin sprouts is affected with net necrosis. If necrosis of this type is at all severe in the tuber, the sprouts are almost certain to be weak and spindling.



FIG. 4. Tuber unit from necrotic tuber. The two hills at right are from basal eyes. All hills exhibited typical leafroll.

SUMMARY

The writer has endeavored to point out the facts that the term net necrosis has an accepted significance in the United States as applying to the browning and dying of the phloem tissues of the tuber, both outside and inside the cambium; that this necrosis has been found to be associated with leafroll, to the extent that necrotic tubers consistently produce leafroll plants and that net necrosis does not persist in leafroll stock but is believed to be a result of initial leafroll infection.

Both leafroll and net necrosis are generally associated with the production of spindling sprouts. Tubers seriously affected with necrosis will invariably produce spindling sprouts from a part or all of the eyes. If normal sprouts are produced by necrotic tubers, they will come from the eyes at the bud end, for the tissues in this region are seldom necrotic, but all plants produced will have symptoms of leafroll.

If the correlations between a potato tuber necrosis and aucuba mosaic, as described by Atanasoff, are substantiated, this will apparently be a second type of tuber necrosis of an infectious nature, the other being the phloem

necrosis associated with leafroll. The causal factors in connection with these diseased conditions are unknown.

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A STUDY OF THE FUNGOUS FLORA OF THE NODAL TISSUES OF THE CORN PLANT¹

C. L. PORTER

The nodal tissues of corn stalks often are found to be decomposed, even though the other parts of the plant may not be visibly affected. Such decomposition may be due either to microorganisms that are definitely parasitic or to other causes. Hoffer and Carr² and Hoffer and Trost³ have quite conclusively demonstrated that, under certain field conditions, organic compounds of iron and aluminium accumulate in the nodal tissues of the corn plant. These accumulations interfere seriously with certain physiological activities of the plant, especially the translocation of foods and the conduction of food materials. As a result, the supply of foods to the root is interrupted and the whole plant is weakened. The leaves arising from affected nodes become fired, and eventually they are completely killed.

The firing of the lower leaves of the corn plant is a matter of common observation. The dead tissues of these leaves are invaded by various saprophytic fungi and bacteria. The organisms growing along and within these tissues arrive at and invade the tissues in the nodal region. The metal compounds account also for the killing of the roots, and thus another channel is provided for the entrance of saprophytes to the internal basal tissues. Such relations have been suggested by Hoffer and Trost.³

If the decomposition of nodal tissue is to be accounted for by the activities of definite parasites, such parasites should be isolated frequently from these tissues. On the other hand, if the organisms found in these tissues are secondary invaders, coming through the dead leaf tissues, a considerable variety of ordinary saprophytes should be found.

A study that would furnish reliable data toward the solution of such a problem would require the examination of a large number of nodes. This opportunity was provided during the summer of 1926. During this time

¹ Cooperative investigation of the Corn Disease Division of the Botany Department of the Agricultural Experiment Station and the Department of Biology, Purdue University.

² Hoffer, G. N., and R. H. Carr. Accumulation of aluminium and iron compounds in corn plants and its probable relation to root rots. I. Jour. Agr. Res. 23: 801-823. 1923.

³ Hoffer, G. N., and J. F. Trost. Accumulation of iron and aluminium compounds in corn and its probable relation to root rots. II. Jour. Amer. Soc. Agron. 15: 323-331. 1923.

the Purdue Agricultural Experiment Station made a survey of the corn-producing areas of the East, South, and Middle West. This survey was for the primary purpose of determining the nutrient needs for corn in these areas by making chemical tests of the stalks. Portions of the corn stalks also were sent to the laboratory from each field surveyed. These stalks were cut off at the surface of the ground and consisted usually of five or six internodes. The age of the corn plants varied from the early roasting ear stage to full maturity. No plants were examined after freezing weather began.

It was my opportunity to culture the nodal tissues of the stalks sent to the laboratory. Stalks from approximately every fifth field surveyed were cultured. Cultures were made from stalks received from the following states: Alabama, Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New York, Ohio, Oklahoma, Pennsylvania, South Dakota, Tennessee, Texas, Virginia, and Wisconsin. These stalks came from fields embracing many different soil types, fertility needs, and physical conditions, as well as a wide geographical and climatic range (Fig. 1).

The aims of the investigation set forth in this paper are: (a) to determine the flora resident in the nodal tissues of corn plants; (b) to ascertain whether the breakdown of nodal tissue is more frequently associated with the chemical and physical condition than with the presence of certain

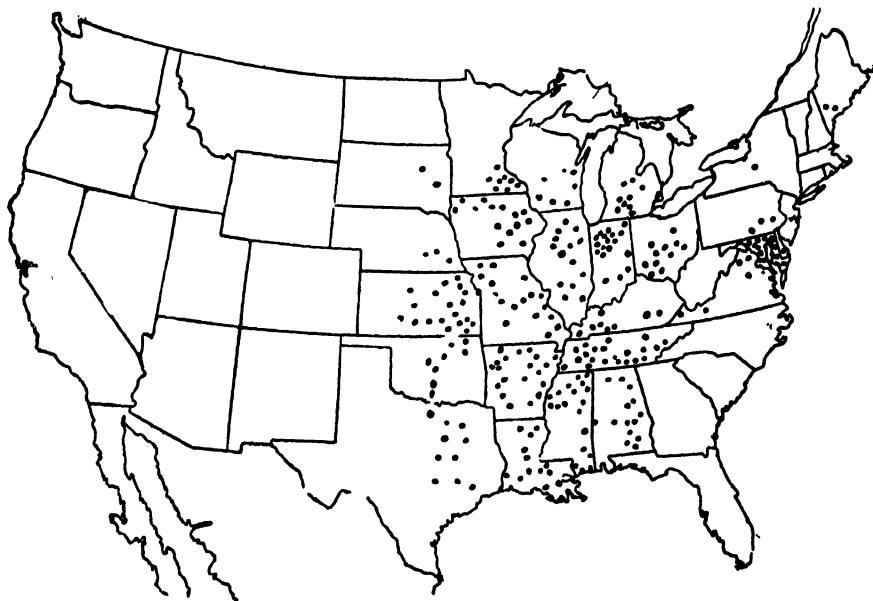


FIG. 1. Map showing the localities from which corn stalks were examined.

specific organisms; (c) to determine if the chemical reserves of the stalk tissues have any appreciable effect on the kind of organisms present.

As soon as the stalks were received in the laboratory, they were split lengthwise. Half of the stalk was tested for the presence of nitrates, potassium, and iron. The methods used for making these tests are those described recently by Hoffer.⁴ The color and state of decomposition of each of the nodal tissues tested were also recorded. A thick layer of the nodal tissues was removed from the other half-stalk with a sterile knife. This procedure exposed a sterile surface. Small pieces from each of the first four nodes were plated on potato dextrose agar under aseptic conditions in an inoculating chamber. For a time, pieces from opposite sides of the same node were plated to discover if there might be a difference in the growths resulting

TABLE 1.—*Organisms isolated from nodes of corn stalks collected from various parts of the United States in 1926. Arranged according to their relative percentage frequencies in isolations*

Organisms	No. of times isolated	Percentage of total no. of isolations
Type I Bacteria	659	28.30
Type III do	266	11.60
Mucor spp.	235	10.20
Fusarium spp.	232	10.05
<i>Fusarium moniliforme</i>	212	9.20
Type IV Bacteria	190	8.30
Penicillium spp.	141	6.10
<i>Rhizopus nigricans</i>	93	4.06
<i>Aspergillus niger</i> group	64	2.80
Yeasts	55	2.40
Type V Bacteria	49	2.10
Type VII do	47	2.05
<i>Alternaria</i> spp.	39	1.70
Type IV Bacteria	35	1.50
<i>Diplodia zeae</i>	28	1.20
<i>Aspergillus glaucus</i> group	19	0.83
<i>Cephalosporium acremonium</i>	17	0.70
<i>Cephalothecium roseum</i>	10	0.41
Type II Bacteria	7	0.35
Unidentified	7	0.35
Type VIII Bacteria	5	0.22
<i>Helminthosporium gramineum</i>	3	0.13
<i>Aspergillus tamaraii</i> group	2	0.09
<i>Cylindrocephalum</i> sp.	2	0.09

⁴ Hoffer, G. N. Testing corn stalks chemically to aid in determining their plant food needs. Purdue University Agr. Exp. Sta. Bul. 298: 1-31. 1926.

from such bilateral plating. The two sides checked so frequently, however, that this line of procedure was abandoned as unnecessary. The total number of platings made was 2,097, and the total number of isolations was 2,288. Table 1 gives a tabulated list of organisms.

The average time that elapsed from the taking of stalks in the field until they were received in the laboratory was 48 hours. In order to determine the effect of holding stalks some time before plating, corn stalks were removed from a field near the Station and plated at once. Stalks from the same hills were wrapped in paper and held in the laboratory 72 hours before plating. In every case the two platings checked closely enough to indicate that delay in plating was a factor of very slight importance.

The types of bacterial organisms as given in table 1 are based on purely morphological characters. Each type is doubtlessly made up of a number of different species. These types may be characterized as follows:

Type I. Colony round, glistening white. Organisms $0.6 \times 1.0 \mu$.

Type II. Colony dull, dry and wrinkled. Organisms $0.6 \times 1.0 \mu$.

Type III. Colony light yellow. Organisms in chains.

Spore formers with spores centrally located. $1.3 \times 4 \mu$.

Type IV. Colony white, spreading "tree-like." Organisms $2.0 \times 0.7 \mu$.

Type V. Colony egg-yellow, spreader. Organisms cocci. 0.6μ in diameter.

Type VI. Colony glistening white, like the white of egg. Organisms spindle-shaped. $1.3 \times 2.6 \mu$.

Type VII. Colony light-translucent in center and grayish white about border. Border crenate. Tendency to form gas in medium. Short heavy rod. $1.0 \times 0.8 \mu$.

Type VIII. Wrinkled yellow colony. Organisms mixed, consisting of small rods $2.6 \times 6 \mu$ and long rods $14.3 \times .4 \mu$.

Bacteria of these various sorts composed 50.7 per cent of the total organisms isolated.

There was no growth in 266 of the platings, or 12.7 per cent of the total number of platings were sterile. The distribution of sterile platings from the first, second, third, and fourth nodes is shown in table 2.

In table 2, Node I represents the lowermost nodes of the stalks plated. In practically every instance this node was the first visible node of the stalk above the surface of the ground. Nodes II, III, IV respectively are the nodes immediately above the lowermost node plated. Thus, as table 2 indicates, forty-five of the lowermost nodes plated were sterile. This number represents 2.14 per cent of all the platings made from the lowermost nodes. Of the total number of sterile platings made 16.92 per cent were from tissues of the lowermost nodes.

TABLE 2.—*The numbers and percentages of sterile platings from the first, second, third and fourth nodes of corn stalks collected from various parts of the United States in 1926*

Nodes	No. sterile platings	Percentage of total platings	Percentage of sterile plates
I	45	2.14	16.92
II	49	2.33	18.42
III	82	3.91	30.82
IV	90	4.29	33.83

It will be noted by reference to table 2 that the percentage of sterility increased progressively as the more superior nodal tissues of the stalk were tested¹, and that the number of sterile platings from the fourth node above the surface of the ground is exactly twice that from the lowermost node, or Node I.

These sterile nodes are significant especially if any reason may be assigned to their sterility. Hoffer and Carr⁵ have shown that metallic accumulations in the nodes eventually cause the firing and death of the leaves arising from such nodes. It has also been shown that the content of iron and aluminium compounds in the nodal tissues is low when potassium salt reserves are present in the stalk tissues. A check was made of the sterile platings to note if there was any apparent relationship between the sterility of the nodal tissues and the iron and potassium accumulations. The results of this investigation are given in table 3.

TABLE 3.—*The relation of sterile platings to the iron accumulations of the nodes of corn stalks*

Iron accumulations ^a	No. sterile nodes	Percentage sterile nodes
Sub-normal (C -).....	51	19.17
Normal (C).....	145	54.51
(C +).....	40	15.03
Excessive (D -).....	13	4.88
(D).....	7	2.63
(D +).....	2	0.83
Very excessive (E).....	0	0.00
Unknown.....	8	3.00

^a The letters used to denote the iron accumulations are the same as shown in Plate II, Bulletin 298 of the Purdue Agricultural Experiment Station. "C" denotes the quantity of iron in normal stalks; "D" denotes an excessive amount; and "E," the highest iron content found in disintegrated nodal tissues.

⁵ Hoffer, G. N., and R. H. Carr. *Loc. cit.*

Examination of table 3 reveals the fact that nearly 90 per cent of all the sterile nodes carried an iron content that was practically normal, and also that there were very few sterile nodes to be found in stalks where the iron accumulations were much above those found in normal plants.

Stalks sent into the laboratory were tested to determine the presence of a potassium reserve. Potassium inhibits the accumulation of iron at the nodal tissues. A high potassium reserve in the stalk was invariably associated with a low iron accumulation. Of 67 stalks showing a potassium reserve of 1+ or more (slight excess), 50 were sterile. This relation suggests a correlation between potassium reserve, low iron accumulations, and sterility of the nodal tissues.

SUMMARY

A great variety of organisms, mostly saprophytic, constitutes the flora of the nodes of corn stalks.

A higher percentage of sterile platings was obtained from the upper nodal tissues than from the lower ones. This is associated with a greater firing of the lowermost leaves.

Sterile platings were more frequently made from nodes carrying a low iron content.

There was no close correlation between the kind of fungi in the nodal tissues and the soil type or degree of fertility of the soil, with the following exceptions: in plants containing a potassium reserve there was a greater tendency for the tissues to be healthy and sterile; and organisms almost always were found in tissues containing accumulations of iron.

No one organism, or several organisms, can be ascribed as primary agents in causing decomposition of nodal tissues.

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DUST TREATMENTS FOR THE CONTROL OF OAT SMUT IN IDAHO¹

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Tests with dusts for the control of grain smuts were started at the Idaho Agricultural Experiment Station in 1920. These tests dealt, for the most part, with the control of bunt or stinking smut of wheat. The materials used were principally copper carbonate dust, together with a number of proprietary compounds. Of the materials used, copper carbonate has proved to be the most generally desirable disinfectant. This confirms work by other investigators.

Believing that some of these disinfectants might exhibit the same toxic property towards the seed-borne spores of oat smut, we conducted a group of tests in cooperation with the Crop Protection Institute. Some of the results were published in 1926.² It is sufficient to state that none of the dust treatments, nor the wet treatments with proprietary compounds, proved to be as effective in controlling oat smut as the formalin treatments.

No tests were conducted in 1925. Stimulated by the work of Thomas in Ohio, we continued the work again in 1926. These tests were conducted with both hulled and hullless oats. The test included twelve treatments and three untreated checks. Each treatment was replicated three times, each replication consisting of one rod row. Of the twelve treatments, nine were made with various dusts, with considerable emphasis placed upon mercuric chloride in combination with various types of fillers.

Unlike the results obtained by Thomas³ or our own results of 1924, absolute control of smut of hulled oats was effected with copper carbonate, and there was only 0.33 per cent infection in the hullless variety. When mercuric chloride was used in combination with various fillers, in the proportion of one part filler to two parts mercuric chloride, complete control was secured in five out of six treatments. The sixth treatment allowed only 0.09 per cent infection. The indications are that mercuric chloride as a dust is quite effective. To what extent it can be diluted remains to be worked out.

These results are not in accord with the results of Thomas,⁴ who failed to obtain control when kaolin was used as a filler, in combination with mercuric chloride alone, or with combinations of mercuric chloride and various

¹ Approved for publication by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 46.

² Lambert, E. B., H. A. Rodenhiser, and H. H. Flor. The effectiveness of various fungicides in controlling the covered smuts of small grains. *Phytopath.* 16: 393-411. 1926.

³ Thomas, R. C. Effective dust treatments for the control of smut of oats. *Science* 61: 47. 1925.

⁴ Thomas, R. C. Control of smuts of wheat and oats with special reference to dust treatments. *Ohio Agr. Exp. Sta. Bul.* 390. 1925.

copper and nickel salts. We used ordinary Palouse silt loam as a filler. The soil was dried, pulverized with mortar and pestle, sifted through a 200-mesh sieve and mixed with mercuric chloride by hand. The only proportion used was two parts of mercuric chloride to one of filler. Thomas's investigations would seem to indicate that other proportions than the above are not effective.

Although the other dusts used controlled smut to a certain degree, none proved as efficient as either copper carbonate or mercuric chloride.

Table 1 gives the list of treatments and results obtained with both hulled

TABLE 1.—*Results of dust treatments for oat smut control with hulled and hulless oats in Idaho in 1926*

No.	Treatment	Results			
		Hulled oats		Hulless oats	
		Germination in per cent	Smut in per cent ^a	Germination in per cent	Smut in per cent ^a
1	Check. No treatment	99.0	7.52 ^b	87.0	25.76 ^c
2	Formalin (1-320) 5 min. Dip.	95.5	0.00	58.5	0.28
3	Formalin (1-1) spray, Cover 4 hrs.	100.0	1.02	7.5 ^d	0.00
4	Formalin (1-10) spray, Cover 4 hrs.	95.0	0.00	4.5 ^d	0.09
5	Copper, carbonate, 3 oz. per bu.	98.0	0.00	88.5	0.33
6	Copper carbonate, 1 part } 3 oz. Mercuric chloride, 2 parts } per bu.	99.0	0.00	85.0	0.00
7	Check. No treatment	99.5	12.49	83.5	9.60
8	Sulphur, 1 part } 3 oz. Mercuric chloride, 2 parts } per bu.	99.5	0.09	90.5	0.00
9	Palouse silt loam, 1 part } 3 oz. Mercuric chloride, 2 parts } per bu.	99.0	0.00	85.5	0.00
10	Dupont dust no. 49, 3 oz. per bu.	100.0	1.61	84.0	0.40
11	Dupont dust no. 52, 3 oz. per bu.	100.0	2.65	91.0	0.45
12	Semesan, 3 oz. per bu.	98.5	3.71	98.0	1.91
13	Bayer dust, 3 oz. per bu.	98.5	2.64	94.0	2.57
14	Colloidal copper, 3 oz. per bu.	98.0	4.93	97.5	2.32
15	Check. No treatment	98.5	14.45 ^c	93.5	12.87 ^c

^a Average of three replications.

^b Average of one replication.

^c Average of two replications.

^d Much better germination was secured in field and a fair stand resulted.

and hulless oats. It will be noticed that germination of the hulless oats was seriously affected by the formalin treatments.

The tests reported upon here are but the results of one year's work (1926). Further tests are necessary before definite conclusions can be made.

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CHEMICAL HYDRATED LIME.FOR THE PREPARATION OF BORDEAUX MIXTURE¹

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The chief difficulty in preparing bordeaux mixture lies in the proper slaking of the lime. The suspension of the resulting calcium hydroxide and its activity or speed of reaction vary with the degree of dispersion or size of particles, as has been shown by different investigators. Unless the lime is thoroughly dispersed and free from large aggregates, the physical properties of the subsequent bordeaux will be seriously impaired and its efficiency as a fungicide reduced. The removal of coarse particles of lime and of insoluble matter from the milk of lime, by sieving or screening through double thickness of cheesecloth, is advisable in all cases but can not convert a poorly dispersed lime into a highly active product, to say nothing of the waste of material involved.

Everyone will concede that the hand-slaking of lime is hard to control, is laborious, and time-consuming, and that the substitution of commercial hydrated lime promises better and more uniform results provided a suitable product can be obtained at a reasonable cost. Agricultural lime is poorly adapted for the purpose; and ordinary plasterers' or finishing lime, containing 55 to 60 per cent active calcium hydroxide, carries too much inert material to be entirely satisfactory, although, if soaked in water over night before use, it yields a bordeaux of good suspension.

Chemical hydrated lime prepared from selected, high calcium limestone is now produced extensively in various sections of the country and is well suited for the preparation of bordeaux. In some instances the limestone employed contains substantially 99 per cent calcium carbonate and is burned in steel kilns fired by wood, coal, gas, or oil. The slaking may be done either by the batch system or the continuous flow process, and the resultant is generally air-separated (floated) to assure a light, bulky product free from grit. The material is white or yellowish, of fine particles, and of good flow unless damp, and, after proper soaking, of fair suspension and high activity. Data furnished by the manufacturers indicate that the better grades of such products contain from 93 to 98 per cent calcium hydroxide, 1.3 to 3.0 per cent calcium carbonate, and small amounts of ferric oxide, alumina, magnesia, sulphuric acid, silica and hygroscopic moisture. From 96.50 to 99.70 per cent will pass a 200-mesh screen. The bulk probably varies with the air-separation and subsequent treatment. Samples tested

¹ From the department of chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

in the Massachusetts laboratory gave a volume of 2.4 to 3.3 cc. per gram, with an average of about 2.6 cc.* The amount of active base in these samples, calculated as calcium hydroxide, averages about 89 per cent, with a range of 85 to 92 per cent. By active lime is meant that portion which can be readily determined by titration against standard acid to the initial disappearance of color, using thymolphthalein as indicator, thereby excluding coarse and other slowly reacting particles. The deterioration of chemical hydrated lime in paper bags, due to air-slaking (carbonating), is said to be less than that of quicklime in wooden barrels under similar conditions. Most producers ship the hydrate in paper bags holding 50 pounds; but cloth bags of 40 or 100 pounds, cloth and paper bags and wooden barrels of 180 pounds, and friction-top steel barrels are occasionally employed at a higher cost per unit. The cost in paper bags f. o. b. the plant varies from \$10.00 to \$15.00 per ton, and the freight to Amherst in l. c. l. lots is approximately the same amount, depending on the point of shipment.

Nine samples of chemical hydrated lime, representing deposits from Indiana, Missouri, Pennsylvania, Virginia, and West Virginia, were submitted by different manufacturers. Bordeaux 4-4-50 was prepared with each sample and the suspension determined as usual. The results are given in table 1.

TABLE 1.—*Suspension of bordeaux mixture (4-4-50) precipitated with chemical hydrated lime*

Chemical hydrated lime		Suspension in per cent		
Brand	Source	1 hr.	2 hrs.	3 hrs.
Lehigh	Mitchell, Ind.	97.2	93.8	90.4
Speed	Milltown, Ind.	97.9	94.5	91.7
Mississippi	Ste. Genevieve, Mo.	97.9	94.1	90.6
Peerless Special	do	97.4	93.3	90.2
Bell-Mine	Bellefonte, Pa.	96.8	92.7	88.9
Bell-Mine	do	97.7	94.1	90.7
Oranda	Capon Road, Va.	97.7	94.7	91.9
Berkeley	Berkeley, W. Va.	97.2	94.0	91.2
Super	Unknown	97.7	94.7	91.9
Average.....		97.5	94.0	90.8

The quality of chemical hydrated lime depends on the purity of the deposit, the care exercised in mining and selection of the rock, and the chemical control of the manufacturing process. All the samples tested proved satisfactory for the preparation of bordeaux and can be recommended as desirable substitutes for quicklime.

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A STUDY OF GROWTH HABIT AND RUST REACTION IN CROSSES BETWEEN MARQUIS, KOTA, AND KANRED WHEATS¹

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INTRODUCTION

Information regarding the mode of inheritance of certain plant characters is to the plant breeder what good and useful tools are to the skilled mechanic. The more complicated and intricate the problem may appear, the greater is the need for fundamental knowledge of all phases of the problem. The mode of attack may possibly be altered considerably if one has available all of the facts concerning the mode of inheritance of a character, or characters. Crop-improvement programs are vitally concerned with such information. Naturally, then, it is desirable to gather as much information as possible on the genetics of our most important crop plants. The purpose of the present study was to ascertain the mode of inheritance of certain plant characters in wheat in relation to the problem of producing improved varieties of rust-resistant spring wheats.

HISTORICAL REVIEW

Growth Habit

Several previous studies of growth habit have been made, but an exact knowledge of the mode of inheritance of this character is not available. Spillman (33), in 1909, reported that the winter type was dominant over the spring type. Fruwirth (10) cites a report of Tschermak, who stated

¹ The data presented in this paper were obtained in cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agriculture of the University of Minnesota; and submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted June 13, 1927.

² The writer is indebted to Dr. H. K. Hayes, Professor of Plant Breeding, University of Minnesota, for valuable suggestions regarding the methods of conducting the study and in the interpretation of the results; and to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, for suggestions on those phases of the investigation dealing with disease resistance.

that the F_1 plants from a cross between spring and winter wheats overwintered somewhat better than the winter parent and remained dormant throughout most of the summer, when the seed was spring-sown.

Caporn (5), in 1918, mentions that the F_1 from a cross between Polish (spring) and Rivett (winter) was intermediate for time of ripening. An "extensive scatter took place" in the F_2 , and the bulk of the plants were earlier than the late parent.

Vavilov and Kuznetsova (37) appear to be the first to report a clear dominance of the spring character over the winter character in crosses between spring and winter wheats. They found a complicated segregation in the F_2 which was apparently due to the action of several factors. Several of the segregates were homozygous for different periods of heading in the F_3 . The reported ratio in the F_2 was 9.6 early or late spring heading to 1 typical winter or non-heading.

The writer (1), in 1923, found the mode of inheritance of growth habit in a spring-winter wheat cross to be practically identical with that reported by Vavilov and Kuznetsova. There was a dominance of the spring type in F_1 , a complicated segregation in F_2 , and a number of lines in F_3 which were homozygous for various intermediate heading periods, as well as lines which bred true for winter and spring habit. Various types of segregation were observed in F_3 .

Cooper (9) also studied crosses between spring and winter wheats. He found that the F_2 from some of the crosses segregated in the proportion of 13 springs to 3 winters, and in others the ratio was 3 springs to 1 winter. The 13:3 ratio was explained on the assumption that there was present a dominant factor for winter habit and an inhibitor factor of the winter habit which also was dominant. Twenty-three families were grown in the F_3 and not all of these were readily explainable on the same basis as the F_2 hypothesis.

Gaines and Singleton (13) obtained a ratio of 35 spring to 1 winter in the F_2 from a cross between a spring and a winter wheat. Their conclusions from this study are in agreement with those of Vavilov and Kuznetsova, and the earlier report by the writer. There is a dominance of the spring habit over the winter habit, and the inheritance of this character is controlled by multiple factors.

Rust Reaction

Complete reviews of literature have been made in previous publications on the breeding of wheats for resistance to physiologic forms of black stem rust. The present status of the problem and a statement of the mode of attack may be found in the papers published by Hayes and Stakman (19) and Hayes, Stakman and Aamodt (20). As the present paper is a report

on only one phase of the more extensive project of producing rust-resistant strains of common wheat, the literature review will be limited to those studies previously reported which have a direct bearing upon the particular aspects of the problem under consideration.

There are 37 physiologic forms of black stem rust (34), 21 of which have been found in the upper Mississippi Valley. It is desirable to obtain new varieties of common wheat which are resistant to all of these forms in the field. By certain crosses and re-crosses, it was thought such wheats could be produced synthetically. Information regarding certain theoretical considerations on the genetic relationship between the host and the pathogen would be of value in planning an intelligent mode of attack on the problem. Considerable evidence has been obtained to support the belief that a combination of the desirable characteristics of several wheat varieties can be obtained in a single variety. On several occasions (30, 14, 15) it was demonstrated, both in intraspecific and interspecific crosses, that the resistant reaction of two varieties of wheat to different physiologic forms could be combined in a single hybrid variety. Evidence has been obtained also that there may be one or several factor pairs in the host which govern the reaction to a single physiologic form (14, 15, 18, 19, 20, 30). In an earlier report (1), the writer demonstrated that one factor pair governed the reaction to several forms of stem rust in crosses between Marquis, a spring wheat, and Kanred, a winter wheat. It was also shown that this same factor for immunity from rust infection was inherited independently of the factors for spring and winter habit of growth in the two parents.

Immunity is complete resistance. It can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field. The Kanred type of immunity from certain forms is of considerable importance in the breeding work because through its use immunity from eleven physiologic forms can be obtained. The fact that the same factor governs the reaction to all eleven forms simplifies the routine of determining whether a hybrid is immune from or susceptible to all of these forms. ✓ Hybrids from crosses in which this factor is concerned can be inoculated with one of the eleven forms from which Kanred is immune, and from the resulting reaction one may conclude that the hybrid is likewise immune from the other ten forms which are governed by this same factor. ✓ Simply by inoculating the F_3 seedlings in the greenhouse, during the winter, with a form of rust from which Kanred is immune, those segregates which are not homozygous for immunity from all eleven forms can be eliminated. ✓ The bulk of the material is thus considerably reduced and this facilitates the study of the reaction to other forms either in the greenhouse or field. Hayes, Stakman, and Aamodt (20) demonstrated that this factor for immunity is inherited independently of two

factors for resistance contained in Marquillo or, if linked, the linkage relation is not very close.

MATERIALS AND METHODS

Parental Varieties

Four varieties of *Triticum vulgare* Vill. were used as parents in the several crosses studied. Marquis is a hard red spring variety of high quality. It is susceptible to stem rust in the field when grown in the spring-wheat region.

Kota is a hard red spring wheat adapted to the less humid sections of the spring-wheat region. It appears somewhat resistant to numerous physiologic forms of rust under field conditions.

Kanred is a hard red winter wheat of high quality when grown in the winter-wheat region. It is immune from several different physiologic forms of stem rust. In the field, however, when certain other forms are present, it may rust quite heavily.

Hybrid No. 1410 is a selection from the cross between Marquis and Kanred. It is a bearded spring wheat which heads normally from three to four weeks later than Marquis.

Crosses

The progeny of four crosses were used in the present inheritance studies. A report was made by the writer (1), in 1923, of the results obtained from a preliminary study of the F_1 , F_2 , and F_3 hybrids from a cross between Marquis and Kanred. Further studies have been made of growth habit in the F_3 and F_4 . A number of selections from this cross, in which were combined the spring growth habit of Marquis with the immunity of Kanred from certain forms of rust, were bulked for rod row trials of yielding ability and other characteristics. These selections were grown as bulks for three years and then again tested in the greenhouse for their reaction to particular physiologic forms as a check on the determinations made in the F_3 .

The F_3 plants from a second cross between Marquis and Kanred were studied for their growth habit only. The seed from F_2 plants grown at Chico, California, was received by the writer from J. A. Clark, of the Office of Cereal Crops and Diseases, United States Department of Agriculture. The seed of the F_1 plants was sown in the fall at Chico and, owing to the mild climate, all of the plants survived the winter. There was no elimination of the non-hardy segregates. Under these conditions all of the plants headed and produced seed. This material then represents a true random F_2 population for both the spring and winter types. In the presentation

of the results in the discussions which follow, the Marquis \times Kanred F_2 plants and their progeny will be referred to as the "random" material in order to differentiate it from that originating at St. Paul.

A cross between Kota and Kanred was made by V. H. Florell, at Davis, California, in 1919. The F_1 was grown by the hybridizer at the same place in the following year. Some of the F_1 plants were harvested and sent by J. A. Clark to L. R. Waldron at Fargo, North Dakota, where an F_2 was grown from spring sowing in 1921. The writer gratefully acknowledges the receipt of the F_2 material. The inheritance of growth habit in the F_2 , F_3 , and F_4 , and the rust reaction in the F_3 and F_4 were studied in this cross.

A study of the inheritance of rust reaction in a cross between Marquis and Kota wheats was made in 1923 (15). Studies were made in the greenhouse of the reaction of the seedling plants to physiologic forms 19 and 21. In the present study it seemed desirable to learn the reaction of the hybrids from this cross to physiologic form 1.

The fourth cross studied was a backcross of a late-heading F_4 hybrid segregate from Marquis \times Kanred to the Marquis parent. In this cross the F_1 to the F_3 inclusive were studied for growth habit and time of heading.

Characters Studied

Two characters, growth habit and rust reaction, received the chief consideration in this study. Growth habit, as used in these studies, is meant to indicate that general characteristic which differentiates true spring varieties from true winter varieties in their ability to produce heads normally when sown in the field in the spring of the year (Fig. 1). The record of growth habit of each individual plant was made by noting the date of emergence of the first head of each plant. In order to facilitate observations, the seeds were sown at intervals of three inches, in rows one foot apart.

The time during which heading took place was divided into weekly periods. One week from the day on which the first plant produced a head tags were placed on all plants on which one or more heads had emerged. These comprised the first class and included the Marquis checks. One week later tags were attached to all plants which had headed since those of class 1. These constituted the second class. This process was continued until no more plants headed. Those plants which did not head were classed as of true winter type. With the exception of the summer of 1925, all of the winter parent controls failed to head, thereby falling into the same class as the winter hybrids.

The rust studies were made on inoculated seedlings in the greenhouse and on plants growing in the field under an artificial epidemic of black

stem rust. The experiments in the greenhouse with the seedling plants were made with urediniospore cultures of known physiologic forms. The cultures were obtained from E. C. Stakman and M. N. Levine. The first leaf was inoculated when about two inches tall. In the field the plants were grown under an artificial epidemic produced by spraying the plants with a suspension of urediniospores of several different physiologic forms of stem rust.

The histological studies were made in an attempt to find some possible morphological character which might be correlated with the differences in rust reaction of Marquis, Kota, and certain Marquis \times Kota hybrids to physiologic form 1. The primary structural characteristic under observation was the ratio of sclerenchymatous tissue to collenchymatous tissue in seedling leaves and peduncles of the plants. The peduncles were collected when the heads were in the soft-dough stage. Pieces about two inches long, taken one inch below the head, were killed and cleared in aceto-alcohol. The seedling leaves were collected when the rust pustules were well developed (18 days) and then treated in the same manner as the peduncles. Sections about 10–15 μ thick were cut in pith by means of a sliding razor. Safranin and Delafield's hematoxylin were used to differentiate the lignified and cellulose tissues.

EXPERIMENTAL RESULTS

Growth Habit

Factors Involved. The rate of growth of winter wheat, when fall-sown in the field, is retarded by the lower temperatures concurrently with the advance of the season. Klages (24) and Maximov and Poiarkova (27) have shown that when winter wheat is sown in the greenhouse, in the fall of the year, there is one continuous growth curve, with no indication of a period of dormancy. Koernicke (25) states that both spring and winter wheats undergo a pause in the course of development and that it is short in the case of spring wheats and long in the case of winter wheats.

Marquis, Kota and Kanred wheats showed no great difference in the form of growth curves when sown in the greenhouse in October. Kota and Kanred headed at the same time and Marquis only a few days earlier. These results are in agreement with those of Klages and of Maximov and Poiarkova. They demonstrate that the rhythm in development of winter wheat, when fall-sown in the field, is enforced and not voluntary or inherent. These same writers also demonstrated that the exposure of fall-sown wheat to freezing temperatures is not essential to culm and flower formation. They grew a number of varieties that had been exposed to low temperatures in the early stages of development. These plants were later brought into the greenhouse, and they all headed on practically the same date as the unexposed plants.

Winter and spring wheats, when sown in the greenhouse in the late winter or early spring, do not show this same relationship. When spring-sown, Marquis and Kota will show a continuous growth curve and head normally. Kanred, on the other hand, will remain dormant for several months or succumb to the high summer temperatures before heading. Klages (24) likewise found considerable differences between spring and winter wheats in their ability to produce heads when grown in the greenhouse from seed sown in late winter or early spring.

On several occasions cultures of Marquis and Kanred were transplanted to the field early in the spring, so that they were exposed to temperatures several degrees below freezing. After having been exposed in this manner, Kanred headed normally and was only a few days later in heading than Marquis. Kanred remained dormant, however, when transplanted from the greenhouse to the field later in the spring after freezing temperature periods had passed. Marquis transplanted at this time headed normally, as did the cultures of both Marquis and Kanred transplanted earlier. Call and Salmon (4) and Jensen (22) have observed that winter wheat will head normally, if the seed is sown in the late winter or early spring, in those years when the season opens very early and is followed by low temperatures. From these results and those of the writer, it appears that the environmental conditions in the late winter or early spring are such that late-sown (February or March) winter wheat naturally goes into a resting or dormant period, and that an awakening from this dormancy can be brought about by exposure to low temperatures.

The studies on growth habit, of all of the hybrid material reported in this paper, were made on plants grown from spring-sown seed in the field. Numerous check rows both of the spring and winter parental varieties were grown in the experimental plots.

Marquis \times *Kanred*. None of the F_1 plants were grown from spring-sown seed; consequently an accurate determination of their growth habit could not be made. The seed was sown in the fall of the year and, owing to the mildness of the season, only two out of 75 plants were killed during the winter. These plants headed at approximately the same time as Kanred.

All of the seed from the F_1 plants was sown in the spring of the following year. There were 5,253 F_2 plants, of which 442, or 8.4 per cent, were of the winter type. Of the 4,811 plants which headed during the summer, 980 headed in the first period at the same time as the spring parent. In the second weekly period, 1,503 plants headed, which proved to be the modal class for the F_2 population. From here on there was a gradual decrease in the number of plants in each class until the eighth heading period, in which there were only 19. If it were assumed that all of the plants which headed during the summer were spring types, there would be a ratio of approxi-

mately 11 spring types to 1 winter. The distribution of the parental varieties and the F_2 in the various classes for growth habit are shown in table 1.

TABLE 1.—*The growth habit of Marquis, Kanred, and F_2 and F_3 hybrid plants from a cross between Marquis and Kanred*

Material	Weekly heading periods									Total number of plants	Per cent winter types
	1	2	3	4	5	6	7	8	Win-ter		
Marquis	131	0	0	0	0	0	0	0	0	131	0.0
Kanred	0	0	0	0	0	0	0	0	111	111	100.0
F_2 total	980	1503	883	568	417	313	128	19	442	5253	8.4
F_3 1	888 ^a	178	37	14	3	1	1	0	5	1127	0.4
F_3 2	501	282	78	65	42	18	7	10	62	1065	5.8
F_3 3	319	392	214	106	39	33	16	7	41	1167	3.5
F_3 4	163	140	286	178	39	30	12	13	94	955	9.8
F_3 5	63	102	79	118	68	52	5	1	102	590	17.3
F_3 6	13	42	37	38	31	32	10	15	90	308	29.2

^a The model class in each F_3 group is underlined

The segregation in F_2 was rather complex and indicated that several factors were concerned with the inheritance of spring and winter growth habit. The F_2 data are fitted to several theoretical ratios and reproduced in table 2.

TABLE 2.—*Segregation in F_2 of Marquis \times Kanred cross for spring and winter types, and calculation of goodness of fit to 3:1, 13:3, 15:1, and 63:1 ratios*

Theoretical ratio	Type		Deviation	Probable error	Deviation P. E.
	Spring	Winter			
3:1	Observed	4811.0 442.0	871.3	21.2	41.1
	Calculated	3939.7 1313.3			
13:3	Observed	4811.0 442.0	542.9	19.1	28.4
	Calculated	4268.1 984.9			
15:1	Observed	4811.0 442.0	113.7	11.9	9.6
	Calculated	4924.7 328.3			
63:1	Observed	4811.0 442.0	360.0	6.1	59.0
	Calculated	5171.0 82.0			

Odds = very large in all cases.

The odds against the occurrence of deviations as great or greater than the one obtained are very large for all of the ratios. The calculations for all four ratios are presented in order to show that by comparison it is evi-

dent that the theoretical expected 15:1 ratio gives the best fit to the observed ratios. Two of the 173 F_2 plants, which were classified as spring types, were homozygous for winter habit in F_3 . If these plants were truly winter types, which headed due to the favorable conditions for growth late in the season, then a correction should be made accordingly in the F_2 classification for growth habit. One and two tenths per cent, or 58, of the 4,811 plants which headed, should properly be considered as winter types on the basis of their breeding behavior in F_3 . The corrected ratio is 4,753 spring types to 500 winter types. This is a wide deviation from the theoretical expected 15:1 ratio. The calculation of goodness of fit for these numbers gives very large odds against the occurrence of a deviation as great as, or greater than the observed one on the basis of random sampling. Such a ratio could be accounted for by assuming that growth habit is governed by two pairs of factors for spring habit which are dominant over their recessive allelomorphs, the factors for winter habit. The breeding behavior of F_2 plants, representing various heading classes in the F_3 , should throw some light on the correctness of this hypothesis.

Sixty-five F_2 plants were selected in 1922 and studied for their breeding behavior in F_3 . Ten plants were taken from each of the first five heading periods, nine from the sixth, and six from the seventh. In the following year, 1923, 108 additional F_2 plants were selected from the various heading periods for a further study of their breeding behavior in F_3 .

The distribution of the F_3 plants in the various classes for heading was somewhat similar to that of the F_2 . Of the 5,212 plants grown, 394, or 7.6 per cent, were winter types. There is also a close relation between the heading period of the F_2 plants and the percentage of winter types produced by their progeny. The distribution of the plants in F_3 and the percentage of winter types produced in the progeny of F_2 plants belonging to separate heading periods are given in table 1. In the progeny of the F_2 plants belonging to class 1, 0.4 per cent of the plants were winter types; in class 2, 5.8 per cent; in class 3, 3.5 per cent; in class 4, 9.8 per cent; in class 5, 17.3 per cent; and in class 6, 29.2 per cent.

F_2 phenotype and frequency	F_3 genotype and frequency	Behavior in F_3
15 spring	1 AA BB	Breeds true for spring habit
	2 Aa BB	Breeds true for spring habit
	2 AA Bb	Breeds true for spring habit
	4 Aa Bb	15 spring : 1 winter
	1 AA bb	Breeds true for spring habit
	2 Aa bb	3 spring : 1 winter
	1 aa BB	Breeds true for spring habit
	2 aa Bb	3 spring : 1 winter
1 winter	1 aa bb	Breeds true for winter habit

If the two-factor hypothesis suggested in the F_2 is correct, the breeding behavior of the F_3 would be as follows: "A" and "B" are dominant duplicate factors for spring habit; and "a" and "b", their allelomorphs, the recessive factors for winter habit.

The proportions of each type breeding true and segregating in F_3 would be as follows: 7 breeding true for spring habit, 8 segregating for spring and winter types, 1 breeding true for winter habit.

Owing to the failure of the winter types to head, the last group would not normally be expected to appear in the F_3 from the St. Paul material, but would be expected in the random material from Chico.

The F_3 lines were classified according to the three groups mentioned above. As only a certain number of F_2 plants were selected from each of the various heading periods, it was necessary to compute their frequency in these classes on the basis of their actual distribution in F_2 . Such calculations will make it possible to compare the proportions of the true-breeding and segregating F_3 lines with the theoretical proportions.

In the second generation there were 980 individuals in the first heading period, which represents 20.5 per cent of the 4,789 F_2 plants that produced seed. In the F_3 , 173 lines were grown. Twenty and five-tenths per cent, or 35.5 plants, would be the calculated number for class 1 on the basis of the F_2 frequency distribution. The actual number of F_3 lines grown from seed of class 1 F_2 plants was 29. Of these, 28 were pure for spring habit and one was segregating for growth habit. The corresponding proportions for the calculated number of 35.5 would be 34.3 pure for spring habit and 1.2 segregating for growth habit. Computations were made in a like manner for each heading class. The observed and calculated results are drawn up in table 3. The actual number of plants selected in F_2 and grown in F_3 is given in the first column of each group and the number calculated on the basis of the F_2 distribution is given in the second column.

Only one of the F_2 plants selected from class 1 produced progeny in F_3 which segregated into spring and winter types. In this one line there were 78 springs to 5 winters. Beginning with the plants in the second heading period, there was a gradual decrease in the number of lines which were homozygous for the spring character and a corresponding increase in the number of lines heterozygous for spring and winter types as the seventh heading period was approached. No families were homozygous for the spring habit of growth in either the sixth or seventh heading periods. F_3 lines from plants with such late heading periods were either heterozygous for growth habit or homozygous for winter habit. In general, the later the heading period of the F_2 plants, the larger the percentage of winter types to spring types in the F_3 .

TABLE 3.—*The breeding behavior in F_3 of plants belonging to separate F_2 heading groups in crosses between Marquis and Kanred*

F ₂ weekly heading period	Number of F ₃ lines							
	Grown		Pure for spring habit		Segregating for growth habit		Pure for winter habit	
	Actual	Calculated	Actual	Calculated	Actual	Calculated	Actual	Calculated
1	29	35.5	28	34.3	1	1.2	0	0.0
2	29	54.3	11	20.6	18	33.7	0	0.0
3	30	31.8	18	19.1	12	12.7	0	0.0
4	28	20.6	10	7.4	18	13.2	0	0.0
5	30	15.1	5	2.5	25	12.6	0	0.0
6	21	11.2	0	0.0	21	11.2	0	0.0
7	6	4.7	0	0.0	4	3.1	2	1.6
Total	173	173.2	72	83.9	99	87.7	2	1.6

When calculated on the basis of the F_2 frequency, the 173 F_3 lines gave a fairly close fit to the theoretical expected. There were 83.8 homozygous for spring habit, 87.6 segregating for spring and winter habit, and 1.6 homozygous for winter habit. The results obtained are compared with the theoretical expected by the goodness of fit method and reproduced in table 4.

TABLE 4.—*Summarized data from table 3 fitted to a theoretical 7:8:0 ratio*

Group	Expected ratio	Ratio		O-C	(O-C) ²	(O-C) ² C
		Observed	Calculated			
Pure spring	7	83.8	80.7	3.1	9.61	0.119
Segregating	8	87.6	92.3	4.7	22.09	0.239
Pure winter	0	1.6	0.0	1.6	2.56	2.560

P = 0.2350

X² = 2.918

Owing to the presence of the homozygous winter type, the fit is not so close as might otherwise be expected. The two F_3 lines, which were pure for winter habit, were the progeny of very late heading F_2 plants. These may possibly have been winter forms which, under the conditions peculiar to 1922, headed and produced seed. Consequently they were wrongly classified as to growth habit in the F_2 . If this possibility is taken into consideration and a correction made accordingly, the recalculated ratios would give a fit to the theoretical expected with a P value of 0.8591. As bad a result, or worse, is expected on the basis of random sampling in about 86.0 per cent of such trials.

The F_2 plants received from Chico were not tagged individually for heading period; consequently a study of the relation between the heading period of F_2 plants and the breeding behavior of their progeny could not be made in this material. The seed from F_1 plants was fall-sown and, owing to the mild climate, all of the F_2 plants survived and produced seed. The F_3 was grown at St. Paul in 1922, in the same field as the first lot of F_2 Marquis \times Kanred material.

A larger percentage of the F_3 lines from the random F_2 were homozygous for the winter habit than of the F_3 lines from St. Paul. Seven out of 233, or 3 per cent, of the former were pure winter types; while 1.6 out of 173, or 0.9 per cent, of the latter were pure winter types. Such a difference, or even a greater one, was to be expected in view of the fact that the winter types in F_2 had not been eliminated in the random material as they were at St. Paul when they failed to head and produce seed. There is a difference of only 2.1 per cent, which probably indicates that not all of the winter types were eliminated in the F_2 at St. Paul. The pure winter types were probably the chief ones to be eliminated; consequently it may be assumed that the F_3 represented a fair random sample of the spring types at least.

The breeding behavior of 233 F_2 plants, representing a true random sample, was studied in F_3 . There were 120 of these F_3 lines homozygous for spring habit, 106 segregating for spring and winter types, and 7 homozygous for winter habit. The data obtained from this material are compared with the theoretical expected 7:8:1 ratio by the goodness of fit method and reproduced in table 5.

TABLE 5.—*Segregation in F_3 of Marquis \times Kanred cross from a random F_2 for spring and winter habit and calculation of goodness of fit to a 7:8:1 ratio*

Group	Expected ratio	Ratio		O-C	(O-C) ²	(O-C) ² C
		Observed	Calculated			
Pure spring.....	7	120	102.2	17.8	316.84	3.100
Segregating	8	106	116.8	10.8	116.64	0.999
Pure winter.....	1	7	14.5	7.5	56.25	3.879
P = 0.0186				X ² = 7.978		

A study of the data seems to indicate that the primary reason for the poor fit is the lack of winter types in the third group. If the conditions peculiar to that year were especially favorable for heading, some of the winter types may possibly have headed. The result would be that the third group containing the pure winters would be too small and the first group containing pure springs would be too large. The deviations of the

observed from the expected seem to indicate that such was the case; consequently a P value as small as 0.0186 was anticipated in the calculations.

In order to obtain an indication of the nature of the segregation in the heterozygous F_2 plants, each segregating F_3 line was classified as a 15:1 or a 3:1 ratio on the basis of its goodness of fit. The tables of probable errors of Mendelian ratios prepared by the Department of Plant Breeding of Cornell University were used for this purpose. The number of individuals in each class for heading was computed on the basis of the frequency distribution in F_2 . The data obtained from these observations and calculations are given in table 6. The observed numbers are given in the first column and the calculated numbers in the second column for each of the ratios.

TABLE 6.—*The breeding behavior in F_3 of heterozygous plants belonging to separate F_2 heading groups in crosses between Marquis and Kanred*

F ₂ weekly heading period	Total number		Number of F ₃ segregating for a				Percentage segregating 15:1
			15:1 ratio		3:1 ratio		
	Actual	Calculated	Actual	Calculated	Actual	Calculated	
1	1	1.2	1	1.2	0	0.0	100.0
2	18	33.7	17	31.8	1	1.9	94.4
3	12	12.7	10	10.6	2	2.1	83.3
4	18	13.2	7	5.1	11	8.1	38.9
5	25	12.6	7	3.5	18	9.1	28.0
6	21	11.2	3	1.6	18	9.6	14.3
7	4	3.1	0	0.0	4	3.1	0.0
Total	99	87.7	45	53.8	54	33.9	

The calculated number of F_3 lines from the St. Paul material segregating for growth habit is 87.7. There are also 106 such lines in the random material, making a total of 193.7 F_3 lines segregating for spring and winter habit. Of these combined numbers, 98.8 gave 15:1 ratios and 94.9 gave 3:1 ratios. According to the hypothesis suggested, one-half of the heterozygous F_3 lines should be segregating for two factor pairs and give 15:1 ratios, and the other half for a single factor pair and give 3:1 ratios. The results are very close to the expected and are fitted to a theoretical 1:1 ratio in table 7.

The observed results produced a very close approximation to the calculated as is indicated by the fit to the 1:1 ratio.

Another method of analysis was used which helped to bring out the nature of the results. This was a comparison of the observed ratios of individual plants with the calculated in the heterozygous F_3 lines. There

TABLE 7.—*Segregation in heterozygous F_3 lines of Marquis \times Kanred cross for 15:1 and 3:1 ratios of spring to winter types and calculation of goodness of fit to a 1:1 ratio*

Ratio class	Number		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
15:1	98.8	96.8	2	4.7	0.4	less than 1:1
3:1	94.9	96.8				

were 970 plants in the F_3 lines from the St. Paul material segregating into 3:1 ratios for spring and winter habit, and 1,988 in those segregating into 15:1 ratios. In the random sample there were 1,698 in the 3:1 ratios and 1,384 in the 15:1 ratios. The results are summarized in table 8, and fitted to their respective theoretical calculated ratios.

TABLE 8.—*Ratios of individual plants in heterozygous Marquis \times Kanred F_3 lines segregating for spring and winter habit and their fit to the theoretical calculated*

Expected ratio	Classes	Number		Dev.	P.E.	D/P.E.	Odds
		Observed	Calculated				
<i>St. Paul F_3 material</i>	3:1			4.5	9.1	0.5	Very small
	Spring	732	727.5				
	Winter	238	242.5				
	15:1			16.8	7.3	2.3	7.3 to 1
	Spring	1847	1863.8				
	Winter	141	124.2				
<i>Random F_3 material</i>	3:1			15.5	12.0	1.3	1.6 to 1
	Spring	1258	1273.5				
	Winter	440	424.5				
	15:1			7.5	6.1	1.2	1.4 to 1
	Spring	1290	1297.5				
	Winter	94	86.5				

In all cases the odds against the occurrence of deviations as great or greater than the observed ones are very small and indicate a good fit between the observed and theoretical calculated ratios.

Early Versus Late Heading. There is a rather close relationship between the heading period of individual plants in the F_2 and that of their progeny in F_3 . The progeny of F_2 plants from class 1 had a mode for heading in class 1. The progeny from class 2 plants, likewise, had a mode in class 1 but to a less marked degree. Those from class 3 F_2 plants had a

mode in class 2; class 4 in class 3; and class 5 in class 4. The F_2 plants from class 6 produced progeny which were rather evenly distributed over all classes of heading in F_3 with a mode in the winter group. The heading periods of the F_2 plants and their progeny are given in table 1.

A complete analysis of early and late heading could not be made for all classes, owing to the failure of the winter types to head and also because of the possible influence of the factors for growth habit upon early or late heading.

From a study of the data presented in table 6, it will be noted that there is a fairly close relationship between the heading period of the F_2 plants and their breeding behavior for growth habit in F_3 . Those plants in the earlier classes, especially in classes 1 to 3, segregated in the majority of the cases into 15:1 ratios, while the plants in the later classes segregated into 3:1 ratios. It appears that when both of the factors for spring habit are present the heading period is, in general, earlier than when only one of the factors for spring habit is present. This relationship is illustrated by the percentage of F_3 lines segregating for a 15:1 ratio and those segregating for a 3:1 ratio. In class 1 there was only one heterozygous line and it segregated into a 15:1 ratio; in class 2, 94.4 per cent segregated into 15:1 ratios; in class 3, 83.3 per cent; in class 4, 38.9 per cent; in class 5, 28.0 per cent; in class 6, 14.3 per cent; and in class 7, 0.0 per cent. In the last named class there were four heterozygous lines and all of them segregated into 3:1 ratios. These results suggest that there are factors for early and late heading in addition to the factors for growth habit. This is illustrated by the cases where rather early heading is combined with a single factor difference for growth habit. The F_2 plant headed early, and its progeny produced a monohybrid ratio for spring and winter habit. On the other hand, some of the F_2 plants which headed as late as the sixth heading period produced progeny which segregated as dihybrids for spring and winter habit. (See table 6.)

A study of early and late heading in the F_2 and F_3 data failed to reveal any especially significant ratios which might be satisfactorily explained on the basis of a single factor for early and late heading in addition to the factors for spring and winter growth habit. However, if it were assumed that there are two factor pairs for earliness and that they are independent of the growth habit factors, a fairly satisfactory fit to a theoretical 189:67 ratio can be obtained. When all four factors are present, the plant might be expected to head as early as the first or second period, and with any one of the four absent and its allelomorph for lateness or winter habit present, the plant might be expected to head as late as the third or fourth period. Considering, then, the F_2 plants in classes 1 to 4 as the earlier group and

those in the remaining classes as the later group, a fairly close approach to the theoretical expected is obtained. An outline of the factorial hypothesis would be as follows in F_2 :

Numerical proportions	Genotype	Phenotype
81	A B C D	Early spring 189 early
27	A B C d	
27	A B c D	
27	A b C D	
27	a B C D	
9	A B c d	Late spring
9	A b C d	
9	A b c D	
9	a B C d	
9	a B c D	
9	a b C D	Early winter
3	A b c d	Late spring
3	a B c d	
3	a b C d	Late winter
3	a b c D	
1	a b c d	

The F_2 phenotypic ratio of early and late heading plants is reproduced in table 9 and calculated for a goodness of fit to a theoretical 189:67 ratio.

TABLE 9.—Segregation in F_2 for early and late heading in crosses between Marquis and Kanred, and calculation of goodness of fit to a 189:67 ratio

Heading class	Ratio		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Early 1-4.....	3934	3878	56	21.2	2.6	11.6:1
Late 5-9.....	1319	1375				

The odds against the occurrence of a deviation as great, or greater than the one obtained on the basis of random sampling are 11.6:1. The four-factor hypothesis, therefore, explains the results in a satisfactory manner. Further study is necessary to substantiate this hypothesis.

A number of F_3 plants were selected from both homozygous and heterozygous lines in the first six heading periods for a study of breeding behavior in F_4 . The results are given in table 10 where the plants are classified according to their heading period in F_3 , the breeding behavior of the F_3 line from which they were selected, and the segregation in F_4 .

Of the 37 plants selected from F_3 lines homozygous for the spring character, 36 produced F_4 progeny which were likewise homozygous for the

TABLE 10.—*The growth habit of F_4 lines from plants belonging to separate F_3 heading groups from a cross between Marquis and Kanred*

F_3 weekly heading period	Growth habit of F_3 lines	Number of F_4 families			
		Grown	Homozygous for spring habit	Heterozygous for growth habit	Homozygous for winter habit
1	Homozygous spring	8	8	0	0
2	Homozygous spring	9	9	0	0
2	Heterozygous	10	6	4	0
3	Homozygous spring	5	5	0	0
3	Heterozygous	10	7	3	0
4	Homozygous spring	10	10	0	0
4	Heterozygous	10	7	3	0
5	Homozygous spring	6	5	1	0
5	Heterozygous	7	5	2	0
6	Heterozygous	6	5	1	0
Total....		81	67	14	0

spring character. One of the F_3 plants selected from the fifth heading period produced progeny which segregated into 15 spring types to one winter type.

There were 43 plants selected from F_3 lines heterozygous for growth habit, and 30 of these produced progeny which were homozygous for growth habit in F_4 . Thirteen were heterozygous for growth habit, ten of which produced 15:1 ratios and three of which produced 3:1 ratios for spring and winter types.

The proportion of spring types to winter types in F_4 was much greater than in F_3 . This is undoubtedly the result of selection in F_3 . In the heterozygous lines, plants with the larger number of factors for spring habit would be most likely to mature seed and consequently the winter types would be expected to decrease proportionally by selection.

Kota \times *Kanred*. The F_2 plants from the *Kota* \times *Kanred* cross, grown at Fargo, North Dakota, were not studied individually for period of heading. The plants were pulled at harvest time and then sorted into spring and winter types. There were 1,305 plants, 1,177 of which headed and 128 of which did not head. This proportion of spring to winter types is very close to that obtained in the F_2 from the *Marquis* \times *Kanred* cross grown at St. Paul, Minnesota. Of the former, 9.8 per cent of the F_2 plants were winter types; and of the latter, 8.4 per cent of the F_2 plants were winter types. The data from the *Kota* \times *Kanred* cross are reproduced in table 11 and fitted to a theoretical 15:1 ratio.

TABLE 11.—*Segregation in F_2 of Kota \times Kanred cross for spring and winter types, and calculation of goodness of fit to a theoretical 15:1 ratio*

Class	Ratio		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Spring	1177	1223.4	46.4	5.9	7.86	Very large
Winter	128	81.6				

On the basis of random sampling the odds against the occurrence of a deviation as great or greater than the one observed are very large. This is a slightly better fit than that obtained from the Marquis \times Kanred cross, however, where the deviation divided by the probable error was 9.6. Calculations of goodness of fit to theoretical 3:1, 13:3 and 63:1 ratios were also made as in the previous cross, but here again the 15:1 ratio gave the best fit between the observed and theoretical expected ratios.

The plants were not classified as to weekly heading periods in the F_2 , as in the former cross. Thirty-nine plants, all of the progeny that produced seed from a single F_1 plant, were grown in the F_3 . Nineteen of the F_2 plants proved to be pure for spring habit in F_3 , and twenty segregated for both spring and winter types. No pure winter types were found. The same factor hypothesis is being used to explain the breeding behavior of the hybrid progeny in this cross as in the cross between Marquis and Kanred. The data are reproduced in table 12 and fitted to a theoretical 7:8:0 ratio.

TABLE 12.—*The breeding behavior of F_3 plants representing a random F_2 population from a cross between Kota and Kanred, and calculation of goodness of fit to a theoretical 7:8:0 ratio*

Group	Expected ratio	Ratio		O-C	(O-C) ²	$\frac{(O-C)^2}{C}$
		Observed	Calculated			
Pure spring.....	7	19	18.2	0.8	0.64	0.035
Segregating	8	20	20.8	0.2	0.04	0.002
Pure winter.....	0	0	0.0	0.0	0.00	0.000
P = very large				$\chi^2 = 0.037$		

On the basis of random sampling such a variation would be expected in about 90 per cent of such trials. These results indicate that the two-factor hypothesis giving a 15:1 ratio in the F_2 is probably the correct one.

The 20 plants segregating in F_3 for both spring and winter types were analyzed for the proportions of individuals producing 15:1 ratios to those producing 3:1 ratios. There were 9 of the former and 11 of the latter.

This is very close to the expected 1:1 ratio and indicates that the observed results are in close agreement with the theoretical expected.

A comparison in this cross was made also of the observed ratios of individual plants with the expected ratios in the heterozygous F_3 lines. There were 517 plants in the F_3 lines segregating for 3:1 ratios for spring and winter habit and 689 in those segregating for 15:1 ratios. The results are summarized in table 13 and fitted to their respective theoretical expected ratios.

TABLE 13.—*Ratios of individual plants in heterozygous Kota \times Kanred F_3 lines segregating for spring and winter habit and their fit to the theoretical calculated*

Expected ratio	Classes	Number		Dev.	P.E.	D/P.E.	Odds
		Observed	Calculated				
3:1	Spring	395	387.8	7.2	6.6	1.1	1.2 to 1
	Winter	122	129.2				
15:1	Spring	639	645.9	6.9	4.3	1.6	2.6 to 1
	Winter	50	43.1				

On the basis of random sampling the odds against the occurrence of deviations as great or greater than the observed ones are very small for both ratios and indicate a good fit between the observed and theoretical expected ratios.

The results obtained from a study of the cross between Kota and Kanred are in close agreement with the results obtained in the study of inheritance of growth habit in the cross between Marquis and Kanred. The two-factor hypothesis fairly satisfactorily explains the breeding behavior. Marquis and Kota may both be designated as having the two dominant factors for spring habit, "A" and "B," and Kanred their recessive allelomorphs for winter habit, "a" and "b."

Marquis \times (Marquis \times Kanred). An attempt was made to throw some light on the genotypic constitution of one of the late-heading hybrid selections by backcrossing it to Marquis, the spring parent. Such a late-heading spring type would probably contain at least one of the factors for spring habit (A or B) and the recessive factors for late heading (a and b).

The F_4 Marquis \times Kanred spring hybrid number "1410" which has a heading period four weeks later than Marquis was used as the hybrid parent in this cross. It was pure for spring habit in F_3 and has not thrown any winter types in the four years it has been grown (Fig. 1). The heading date of each individual plant was noted and classified as in the Marquis \times Kanred cross. The data thus obtained are presented in table 14.

There were 104 F_1 plants grown in the field. They all headed in the same weekly period as the Marquis parent, indicating a complete dominance of the early over the late heading. The genotype of Marquis may be designated as AA BB CC DD, and that of the hybrid selection may be designated

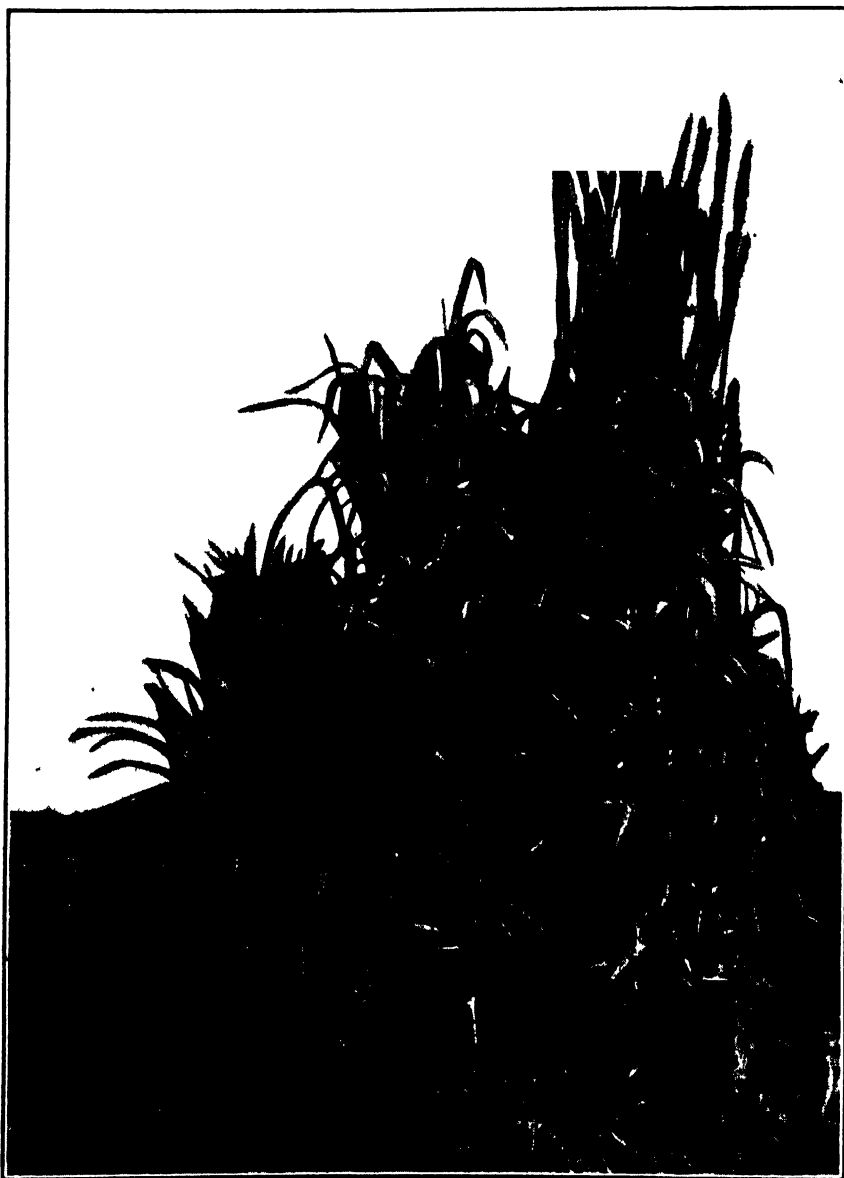


FIG. 1. The growth habit of Kanred, Marquis \times Kanred hybrid 1410, and Marquis at the time Marquis was fully headed.

TABLE 14.—*The growth habit of Marquis, Kanred, Hybrid 1410 and F₂ and F₃ hybrids from a backcross between Marquis and Hybrid 1410*

Year	Material	Weekly heading periods							Total number of plants
		1	2	3	4	5	6	Winters	
1924	Marquis	55	3	0	0	0	0	0	58
	Kanred	0	0	0	0	0	0	13	13
	Hybrid 1410	0	0	5	17	7	4	0	33
	F ₁ total	104	0	0	0	0	0	0	104
	F ₂ total	43	94	24	5	1	0	127	179
1925	Marquis	359	48	0	0	0	0	0	407
	Kanred	0	0	0	0	1	6	171	178
	Hybrid 1410	0	14	139	135	21	0	0	309
	F ₂ total	3184	2774	682	78	2	0	0	6720
	F ₃ 1	772	306	27	2	0	0	0	1107
	F ₃ 2	353	807	311	48	11	0	0	1530
	F ₃ 3	2	27	24	6	0	0	0	59
	F ₃ 4	0	4	31	10	0	0	0	45
	F ₃ 5	0	5	25	9	0	0	0	39

as AA bb cc dd. The F₁ will then be AA Bb Cc Dd, and would be a spring type with an early heading period.

A number of F₁ plants were grown to maturity in the greenhouse during the winter of 1923-24. The seed from these plants was late in maturing and when sown in the field produced plants which grew poorly. They appeared to be rather unsatisfactory material upon which to make a study of growth habit and heading.

The following summer, an F₂ population of 6,720 plants was grown from seed produced by the F₁ plants raised in the field. Of this large number, not one remained dormant as a true winter wheat. The season was unusual, as was indicated by the heading of several of the Kanred check plants. This was the first instance in the five years during which these studies were carried on that any of the Kanred plants headed when grown from spring-sown seed. Under such conditions it is rather difficult to conclude whether any true winter types were produced by the backcross. There is an overlapping in the frequency distribution of the hybrid plants and Kanred in class 5. One Kanred plant headed at that time, and six headed in the sixth period. Seed from the Kanred plants that headed was harvested and sown in the fall. The plants survived the winter and appeared to be typical of the variety.

Owing to the heading of some of the winter parent plants, it seemed that probably the season was unusually favorable for the development of this character. The variability of the parental varieties in this particular

year is so great that one could scarcely expect to find the hybrids fitting into any particular classification. Marquis plants headed over a period of two weeks, the late spring hybrid over a period of four weeks, and Kanred over a period of at least three weeks. This great variability may possibly be accounted for by the flooding of that portion of the field in which this material was grown, on two separate occasions, the first one at the time the plants were beginning to send out shoots and the second during the first weekly heading period. The F_3 plants, which were the progeny of the F_2 plants grown in 1924, were also subject to these same conditions and consequently not amenable to a detailed analysis from which very definite conclusions can be drawn. The summer of 1925 was an illustration of how erratic the environmental conditions can be in their influence upon such characters as time of heading and growth habit. In general, the results do seem to show that a large proportion of the segregates in the F_2 were of the earlier group and that probably no true winter types were produced in the F_2 or F_3 progeny from this cross.

Seedling Posture. There are distinct differences in the posture of the leaves of various wheat varieties in the early stage of development of the plant. Hardy varieties of winter wheat are commonly believed to have a recumbent habit in contrast to an upright one characteristic of most spring varieties (31). This is generally true, but there are some outstanding exceptions, such as Padui, which has a marked upright leaf posture and is also winterhardy. Dawson's Golden Chaff and Red Cross are reported by Klages (24) as being erect types and yet quite hardy.

The 233 F_3 families representing a true random sample from a Marquis \times Kanred cross were studied for this character in the field. The plants were grown from spring sowings. The notes on posture were made early in June and recorded in five classes or types. The leaves of Kanred, the winter parent, were prostrate and the plants were designated as "type 1." The leaves of Marquis, the spring parent, were erect and the plants were designated as "type 5." Intermediate degrees of erectness were designated as types "2," "3," and "4." Many of the F_3 lines were still segregating for this character, in which case estimated averages were made to represent the line as a whole. Consequently, it is not known whether the F_3 lines were homozygous or heterozygous for this character.

Later in the season each F_3 line was classified as being either homozygous for spring habit, heterozygous for spring and winter habit, or homozygous for winter habit. The date of heading was not taken on each individual plant. The relationship between the two characters is shown by the amount of association between the plants in the various classes of seedling posture and their distribution in the three groups for growth habit. The data are presented in table 15.

TABLE 15.—*Relation between seedling posture and growth habit in 233 F₃ lines from a cross between Marquis and Kanred*

Material	Growth habit	Seedling posture ^a				
		1	2	3	4	5
Kanred	Winter	4	0	0	0	0
Marquis	Spring	0	0	0	0	16
117 F ₃ lines	Spring	0	3	56	39	19
109 F ₃ lines	Spring and winter	0	18	82	6	3
7 F ₃ lines	Winter	0	4	3	0	0

^a 1 = prostrate; 2 = semi-prostrate; 3 = intermediate; 4 = semi-erect; 5 = erect.

None of the F₃ lines was homozygous for the prostrate condition typified by the Kanred parent plants, while a number of lines had an upright posture similar to that of the Marquis parent. There were several individual plants in some of the lines heterozygous for this character, however, which had a prostrate position similar to that of the winter parent. A few hybrids with a spring growth habit approached closely the posture of Kanred. The hybrids with a winter growth habit were either semi-erect or approached the winter parent in posture. There appears to be only a general relationship between growth habit and seedling posture in the hybrids. Consequently the utilization of this characteristic as a criterion in the determination of spring and winter wheats, even in this cross, could only be in a relative way.

RUST REACTION

Marquis × *Kanred*. The greenhouse study of the inheritance of rust reaction in crosses between Marquis and Kanred was made on the F₃ and F₇ seedlings. In this cross the infection types of the parental material and the hybrids were so distinctly different that to obtain the numerical ratios, after infection, was simply a matter of mechanical assortment and counting of the number of individuals in each class. Macroscopically, at least, there were no essential variations in reaction. The parental material and all of the hybrids were either immune from (type 0) or completely susceptible to (type 4) *Puccinia graminis tritici*, form 1 (Fig. 2). As the parents are at the extremes in the range of infection types for this particular form of rust, no transgressive segregation was observed. The Marquis parent and susceptible hybrid plants produced large, vigorous, and confluent uredinia.

The plants of ten F₃ lines from each of the first six classes and five families from the seventh class for growth habit were tested for their reaction to physiologic form 1. Of the 65 lines tested, 23 were homozygous for

immunity, 32 were heterozygous for immunity and susceptibility, and 10 were homozygous for susceptibility. The F_2 data are reproduced in table 16, and fitted to a theoretical 1:2:1 ratio.

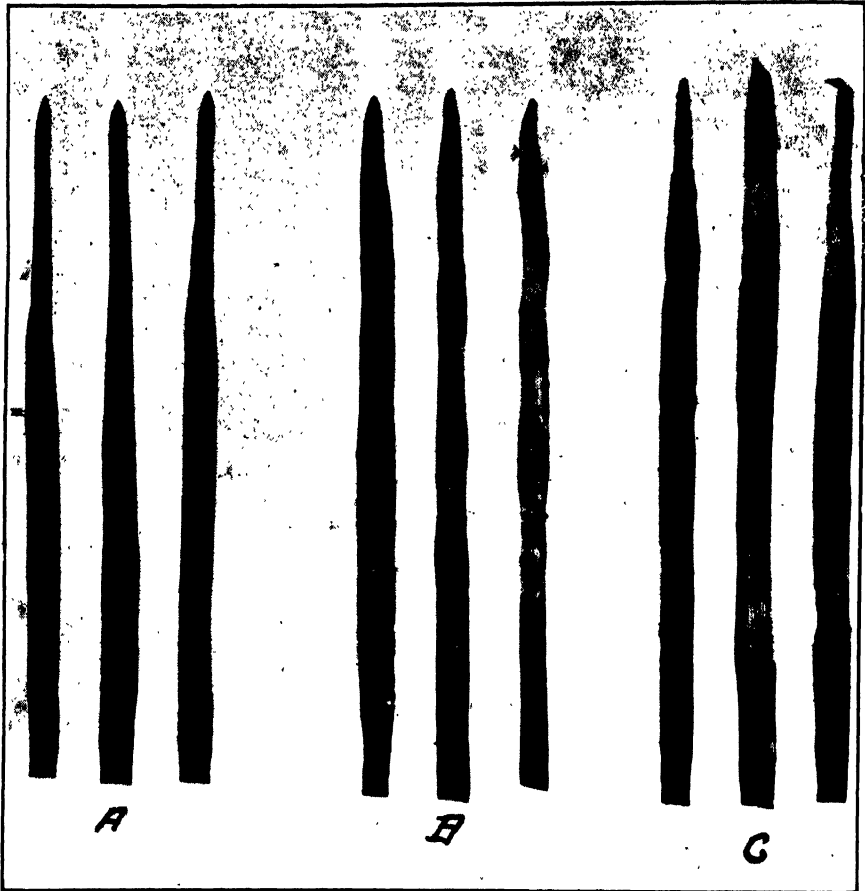


FIG. 2. A multiple allelomorphic series for rust reaction of Kanred (A), Kota (B), and Marquis (C) to *Puccinia graminis tritici* form 1.

As bad a result, or worse, is expected on the basis of random sampling in about 7 per cent of such trials. This fit is not especially close but suggests that the results may be accounted for by a single pair of allelomorphic factors. When the immune and heterozygous groups are combined, there is a close approach to the expected 3:1 ratio. The deviation from the theoretical calculated is 6.2 ± 2.35 . The odds against the occurrence of such a deviation being due to random sampling are 11.58:1. This fit is quite satisfactory and suggests that the results may be accounted for by a single pair of allelomorphic factors, dominant for immunity in Kanred and

TABLE 16.—*Segregation of rust reaction in F_3 of a cross between Marquis and Kanred and calculation of goodness of fit to a 1:2:1 ratio*

Class	F_3 lines		O-C	(O-C) ²	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Immune	23	16.2	6.8	46.24	2.854
Heterozygous	32	32.5	0.5	0.25	0.008
Susceptible	10	16.2	6.2	38.44	2.373
Total	65	64.9			
P = 0.0745			X ² = 5.235		

recessive for susceptibility in Marquis. Additional data on this factorial relationship were made available from a study of the ratios of immune to susceptible plants in the heterozygous F_3 lines.

There were 1,020 plants in the heterozygous F_3 lines, 798 of which were immune from rust and 222 of which were susceptible. This is a deviation from the expected monohybrid ratio of 33.0 ± 9.33 . A number of the plants escaped infection, as was indicated by the susceptible parent used as a check and by reinoculating some of the plants which had escaped infection. The number which had escaped infection was subtracted from the immune group and added to the susceptible group. A corrected ratio of 767 immune to 253 susceptible was obtained. This has a deviation from the theoretical calculated of 2.0 ± 9.33 , which is a very close fit to the expected monohybrid ratio. These results add further proof to the hypothesis that a single pair of genetic factors governs the reaction to physiologic form 1, and that the factor for immunity is dominant to the allelomorphic factor for susceptibility.

The F_3 lines from the different heading periods showed similar numerical relations between the immune and susceptible plants, regardless of their respective times of heading. Rust reaction was independent of growth habit and heading period. Many F_3 and F_4 lines were obtained which are homozygous for the spring habit of growth and immune from infection by form 1. Several of these lines were then studied for their reaction to other physiologic forms of stem rust from which Kanred is immune. The hybrids which were immune from form 1 were likewise found to be immune from the other forms, and it was definitely demonstrated that the immunity of Kanred from several physiologic forms of stem rust was governed by a single pair of genetic factors.

Several hundred of the F_3 lines which appeared to be homozygous for the immune reaction of the Kanred parent were subsequently grown in the field. Fifty-three of these lines which appeared to be desirable spring

wheats were later given an extensive test in the rod row nursery. In the seventh generation they were again grown in the greenhouse and a determination made of their reaction to several of the same physiologic forms of stem rust used previously. Kanred is immune from five of these forms, namely, 1, 9, 17, 19, and 21, and susceptible to forms 3 and 18. Marquis is susceptible to all of these forms, except 19, to which it is resistant. Each hybrid line was given three trials by inoculating three separate sets of plants with each form. There were 12 to 15 seedlings in each set, making an average of 40 seedlings per line.

Fifty of these lines were immune from forms 1, 9, 17, 19, and 23. Two were heterozygous for immunity and susceptibility, and one was susceptible. Approximately 10,000 seedlings were inoculated with the five forms from which Kanred is immune. Of this number, 11, or 0.11 per cent, were susceptible. These exceptions are probably due to natural crosses with some susceptible variety. If such is the case, the cross must have occurred not later than the fifth generation, as immunity is dominant to susceptibility. Marquis checks were grown in every fifth plat in the rod row experiments in the field. Even though the susceptible parental checks were outnumbered five to one and a correction was made strictly on a numerical basis, there would be one-half of one per cent natural crossing between varieties. The rod rows are carefully rogued each year, thus insuring the elimination of a large number of the natural crosses; consequently this figure is much below the one usually given for wheat (17). There is also the possibility that the presence of some of the aberrant seedlings was due to mechanical mixtures.

There was a ratio of 245 immune plants to 108 susceptible in the two F_7 heterozygous hybrid lines. If the plants were heterozygous at the time the individual plants were bulked for the rod rows, it would be difficult to state what proportions of immune and susceptible plants were combined. If they were immune and a natural cross took place in one of the earlier generations, one would not expect such a large percentage of susceptible plants.

The results further substantiate the belief that the reaction to one of several forms of rust from which Kanred is immune may be taken as a criterion of immunity from the remainder of the forms governed by the same factor.

Kota \times *Kanred*. The inheritance studies of reaction to form 1 in crosses between Kota and Kanred were made from the F_3 seedlings. Kanred is immune from this rust form and Kota is moderately resistant (Fig. 2). While the infection types of these parental varieties were quite distinct and uniform, some of the F_3 hybrids which were supposedly like Kota did fluctuate somewhat and produce a "type 4" infection. The Kota parent

in this particular experiment did not show any such variation in susceptibility, but it has been so reported by other investigators (34). The increased susceptibility of certain hybrids might be accounted for by a factorial recombination which resulted in transgressive segregation or as differences due to environmental fluctuation.

The inoculations were repeated on a second series of plants, and in this case practically all of the hybrids, which had previously given a "type 4" infection, produced a "type 3" infection similar to that of Kota. In only one of the 104 F_3 lines did the plants appear to have a consistently higher degree of susceptibility. Such an F_3 line may possibly be the result of some recombination of minor modifying factors. The evidence available to substantiate such an hypothesis is meager and would be difficult to obtain. One aberrant line out of 104 could readily be accounted for by a natural cross with some susceptible variety in the F_1 . These results seem to indicate that the reaction of some of the Kota \times Kanred F_3 lines was subject to variations in rust reaction due to certain uncontrolled environmental conditions. In summarizing the results from this cross, the few variations of the susceptible hybrids from the typical "type 3" infection were considered as environmental fluctuations.

From each of 104 F_3 lines, 30 seedlings were inoculated with physiologic form 1. Of these F_3 lines, 30 were homozygous for the immune reaction, 48 were heterozygous and 26 were homozygous for the moderately resistant reaction. The F_2 data are presented in table 17 and fitted to a theoretical 1:2:1 ratio.

TABLE 17.—*Segregation of rust reaction in F_3 of a cross between Kota and Kanred and calculation of goodness of fit to a 1:2:1 ratio*

Class	F_3 lines		O-C	(O-C) ²	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Immune	30	26	4	16	0.615
Heterozygous	48	52	4	16	0.308
Moderately resistant	26	26	0	0	0.000
Total	104	104			

$P = 0.5598$

$X^2 = 0.923$

As bad a result, or worse, is expected on the basis of random sampling in about 56 per cent of such trials. Additional data on this factorial relationship were made available from a study of the ratios of immune to moderately resistant plants in the heterozygous F_3 lines.

There were 1,246 individual plants in the 48 heterozygous F_3 lines. Of this number, 939 were immune and 307 were moderately resistant. The

number expected on the basis of a 3:1 ratio would be 934.5 immune to 311.5 moderately susceptible. This is a deviation of the observed from the theoretical calculated of 4.5 ± 10.24 . The odds against the occurrence of such a deviation due to random sampling are less than 1:1. These results add further proof to the hypothesis that a single pair of genetic factors governs the reaction to physiologic form 1 in the cross between Kota and Marquis. The factor for immunity in Kanred is dominant to the allelomorphic factor for moderate resistance in Kota.

The inheritance of reaction to physiologic form 1 in the Kota \times Kanred cross appears to be rather closely related genetically to the inheritance of reaction to this same form of rust in the Marquis \times Kanred cross. In the latter the immunity of Kanred dominated the complete susceptibility of Marquis. In the Kota \times Kanred cross the immunity of Kanred likewise dominated the moderate resistance of Kota. The question naturally arises, are these factors for susceptibility in Marquis and moderate resistance in Kota allelomorphic? A study of the reaction of Kota \times Marquis hybrids to the particular form of rust was made in an attempt to obtain further information on the factorial relationship of these two varieties.

Marquis \times Kota. A study of the inheritance of rust reaction in a cross between Marquis and Kota to physiologic forms 19 and 27 was previously reported by Hayes and Aamodt (15). Seed of the F_2 plants was still available and used in the present study of the inheritance of reaction to form 1.

In most of the studies already reported on the inheritance of rust reaction in wheat to particular physiologic forms of rust, the parental infection types have been distinct enough so that the various recombinations in the hybrids could be determined with a rather high degree of accuracy.

The infection types produced on Kota and Marquis by form 1, however, while quite distinct, sometimes overlap (34). Kota is moderately resistant and has a "type 3" infection which is described by Stakman and Levine (34) as follows: "Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present." Marquis is very susceptible and has a "type 4" infection which is described as follows: "Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable."

Seedlings of 62 F_3 lines from the Marquis \times Kota cross were inoculated with urediniospores of form 1. As expected, the hybrids were difficult to classify. There were various gradations from the "type 3" to the "type 4" of infection and in some cases combinations of the "type 3" and the "type 4" on the same leaf. The parental varieties used as checks were quite distinct in their infection types and showed very little overlapping. Several

of the F_3 hybrids also appeared to be homozygous for infection type. In order to be certain of the determinations of rust reaction the experiment was repeated by inoculating another set of the same material. The majority of the hybrids showed approximately the same reaction as in the first test. On the basis of the combined results from both tests, the F_3 lines were classified as follows: 16 moderately resistant, 39 heterozygous, and 7 very susceptible. The theoretical expected on the basis of a 1:2:1 ratio would be 15.5:31:15.5. The data are presented in table 18 and fitted to a theoretical 1:2:1 ratio by the goodness of fit method.

TABLE 18.—*Segregation of rust reaction in F_3 of a cross between Marquis and Kota and calculation of a goodness of fit to a 1:2:1 ratio*

Class	F_3 lines		O - C	$(O - C)^2$	$\frac{(O - C)^2}{C}$
	Observed	Calculated			
Susceptible	16	15.5	0.5	0.25	0.016
Heterozygous	39	31.0	8.0	64.00	2.065
Moderately resistant	7	15.5	8.5	72.25	4.661
Total	62	62.0			
P = 0.035			X ² = 6.742		

As bad a result, or worse, would be expected on the basis of random sampling in about 4 per cent of such trials. Additional data were made available through a study of the ratios of immune to susceptible plants in the heterozygous F_3 lines.

There were 848 plants in the 39 heterozygous F_3 lines, 501 of which were classed as moderately resistant and 347 as susceptible. In some lines there was a preponderance of the "type 4" of infection, and in others a preponderance of the "type 3," and in still others the two types of infection were about equally frequent. There appeared to be no transgressive segregation in either direction nor types that were consistently intermediate between the two parents.

These results seem to indicate that the factors which govern the reaction of Kota and Marquis to physiologic form 1 are allelomorphous to each other as well as to the factor for immunity in Kanred. The reaction of Marquis, Kota, and Kanred to physiologic form 1 is governed by a series of three multiple allelomorphous factors. The Kanred factor results in an immune reaction, the Kota factor in a moderate resistance, and the Marquis factor in complete susceptibility (Fig. 2).

Host Morphology

The physico-chemical properties of the sap of different wheat varieties have been studied by several investigators and no consistent correlations were found between these properties and rust resistance. Certain internal structures of the host have been shown to determine in some measure the extent and spread of development of the fungus. Extensive studies of the morphology of different wheat varieties and a comprehensive literature review on the subject have been made by Hursh (21). He found that the sclerenchymatous tissue in the stems of certain resistant varieties was very large in proportion to the amount of chlorenchymatous collenchyma. As the rust fungus can live practically only in the collenchyma, it is evident that the amount of collenchyma may possibly limit to a considerable extent the development of rust sori.

Histological examinations were made by the writer, of seedling leaves grown in the greenhouse and stems from plants grown in the field, in an attempt to find some explanation for the different reactions of the plants to rust attacks. Seedling leaves of Marquis and Kota which showed a considerable difference in the size of the rust pustules appeared to have no structural differences which could be correlated with the infection types. Sclerenchymatous tissue was found primarily at the extreme edge of the leaf and next to the vascular bundle of the mid-vein. In both regions the sclerenchyma cells were located just beneath the epidermis.

Seedling plants of the 16 F_3 families homozygous for the "type 3" of infection and the 7 F_3 families homozygous for the "type 4" were again grown in the greenhouse and inoculated with physiologic form 1. The reactions of the plants to the pathogen were in agreement with the first two trials. These leaves were then examined histologically and found to show no outstanding structural differences which might be correlated with the two infection types. The shape of the epidermal cells and the thickness of the cell walls appeared to be associated with infection types in a few cases but they were not consistently so. Likewise, it did not seem that the limited production of sclerenchymatous tissue in the seedling leaves could in any way, at least mechanically, have an influence on the development of the rust sori.

Transverse sections of Marquis and Kota stems from field-grown material were examined histologically and found to show approximately the same structural relationships as reported by Hursh. The collenchymatous areas are confined to regions within the sclerenchymatous tissue. In Kota these areas are small and not continuous. In Marquis the collenchymatous areas are larger and often several were confluent and formed a continuous area of collenchyma. Such coalesced areas were not so numerous in this

variety for the seasonal condition under which the experiment was conducted as in the case reported by Hursh (21).

Stems of the F_3 hybrid lines were examined in this same manner. There appeared to be no apparent differences in the ratios of sclerenchyma to collenchyma between the hybrids which showed a "type 3" infection and those which developed a "type 4." All of the hybrids examined showed development of sclerenchyma intermediate between that of the two parental varieties. When these plants were grown in the field under an artificial epidemic of stem rust, produced by spraying with a large number of different physiologic forms, there were no observable differences in their rust reactions.

Kanred stems also were examined for their structural characteristics. A larger number of the collenchyma areas were confluent in it than in Marquis, and they appeared to be about as in the Marquis described by Hursh. Two Marquis \times Kanred hybrids also were examined for their sclerenchyma-collenchyma ratio. One of these selections was susceptible to form 1, like Marquis, and the other was immune, like Kanred. Both of the hybrids had the same structural characteristics, however, and were quite similar to Marquis.

The histological studies on the structural differences in the seedling leaves and the stems of Marquis, Kota, Kanred, and several hybrids fail to demonstrate any particular relationship between these differences and the reactions of these same varieties and selections to physiologic form 1.

DISCUSSION

The nature of the processes involved in bringing about the heading period of our winter wheats has been a matter of speculation and experiment for some time. The environmental conditions which control these processes and permit winter grains to head and to produce seed are closely interrelated with each other. Some investigators believe that both spring and winter wheats undergo a pause in the course of development and that it is short in the case of spring wheats and long in the case of winter wheats. This is probably true when the environmental conditions are such that growth is more favorable for the spring wheats than it is for the winter wheats. The climatic conditions in the field and greenhouse in the spring of the year appear to favor the spring wheat more than the winter wheats in this respect. The results obtained by the writer with Marquis, Kota, and Kanred and those obtained by Klages with several other varieties seem to demonstrate this fact.

On the other hand, these same varieties showed no great differences in the form of their growth curves when planted in the fall in the greenhouse. The

writer's results are in agreement with those of Klages, and Maximov and Poiarkova in this respect. The conditions for growth in the greenhouse in the fall of the year do not seem to favor the spring wheats any more than the winter wheats. Both wheats respond equally well and show a continuous growth curve with no indication of a period of dormancy. Low temperatures were not necessary to force the winter wheats into continuous growth until flowering.

Winter wheats, when spring-sown, have a tendency to fall into a resting period. Freezing temperatures, however, seem to release the necessary stimulus for a continuous growth curve. Kanred grown from spring plantings in the greenhouse will have a continuous growth curve if transplanted to the field early enough to be subjected to low temperatures, while it remains dormant if left in the greenhouse or transplanted to the field after the freezing temperatures have passed. These results appear to be in agreement with the observations made by Call and Salmon, and by Jensen, that winter wheat will not go into a dormancy period if it is planted early enough in the late winter or early spring. The results indicate that length of day and temperature are both important factors in this complex process. It is quite evident that these variations from the usually observed course of development are probably the result of changes in environment which allow the inherited potentialities of the plants to come to expression. Under low intensities of light, as from fall sowing in the greenhouse, there appears to be little difference in the form of growth curves of spring and winter wheats. Under the higher light intensities and longer daily light duration of the early spring, the spring wheats have a normal growth curve and the winter wheats remain for some time in the vegetative stage. In this latter case it appears as though low temperatures may take the place of light and furnish the necessary stimulus so that winter wheat may produce heads.

After a consideration of the influence of the environmental conditions upon the development of such characters as growth habit and time of heading, it is evident that the data can not be analyzed and interpreted with as much precision as may be desired. The results show that there is a rather complicated relationship in the genetic differences between spring and winter wheats. That several factors are concerned with the inheritance of this character appears to be a common opinion. The influence of the environmental conditions upon the plant's ability to produce heads, without a dormancy period intervening, is sufficient to explain the small differences in results obtained from year to year or place to place. Gaines concluded that he had a rather high proportion of spring to winter types, compared to other investigators, owing to the early planting and the fact that the conditions were such that the winter parent plant headed. A winter wheat grown from spring-sown seed in a region where the days are long is more likely to head

than in a region where the days are short. The influence of such varied climatic conditions upon the growth habit of a wheat demonstrates the impracticability of completely correlating the results of different investigators. The variation in results reported may also be accounted for by wide genetic differences which various wheat varieties may have for this character. The segregation which one would expect in a cross depends upon the factors present for growth habit in the parental material, date of seeding, and the environmental conditions peculiar to the season in which the hybrid plants are grown.

In the cross between Marquis and Kanred, the breeding behavior of the F_2 and F_3 plants indicated that there is a two-factor difference for growth habit between these two varieties. The same relationship holds also for the cross between Kota and Kanred. Marquis and Kota each contain two pairs of factors for spring habit which are dominant to the recessive allelomorphs in Kanred for winter habit. The F_2 produce an approximate 15:1 ratio for spring and winter types. The factorial hypothesis used to explain these results was corroborated as being the most plausible explanation as shown by the breeding behavior of the F_3 .

The data also seemed to indicate that there are at least two factors for early and late heading in addition to the two factors for growth habit. The results are not as conclusive for this character as for growth habit, and further study will be necessary before much certainty can be attached to the nature and number of the factors concerned.

It was suggested that the genotypic constitution of the late-heading Marquis \times Kanred hybrid would be AA bb cc dd. When it is crossed with Marquis, which is probably AA BB CC DD, the F_1 will be AA Bb Cc Dd. It would be an early spring type. No winter forms would be expected in the later segregating generations, as both parents are homozygous for the A factor. There would be a segregation in the F_2 for early and late heading types in an approximate 15:1 ratio. The actual results obtained from this cross fairly well fitted the theoretical expectations according to the above hypothesis.

In order to prove more conclusively the factorial constitution of these late spring types and the hypothesis in general, a study of the type of segregation in intercrosses between several late-heading spring types and with the winter parent would probably throw considerable light on the problem. Two late spring selections, each one containing a different pair of the factors for spring habit, and the reciprocal pair of recessive factors for winter habit, when crossed, ought to produce some winter types in the F_2 . The crosses would be as follows: AA bb \times aa BB. The F_1 factorial composition would be Aa Bb and the plant a spring type. One plant out of every

16 F_2 segregates would contain the recessive factors for winter habit, aa bb. Crosses of this type are contemplated, and theoretically it seems should demonstrate that some plants with winter growth habit can be produced by a cross between two late spring wheats.

In an inheritance study of rust reaction in crosses between Marquis and Kanred it was definitely demonstrated that immunity from several forms of stem rust is governed by a single genetic factor. The present studies on 54 F_7 lines from this cross further substantiated the belief that such was the case. Three of these lines appeared to be a mixture of types for rust reaction due to an error in the first determination in the F_3 . The other 51 lines, however, were practically homozygous for the immune reaction determined previously.

The studies of the inheritance of reaction of seedlings to physiologic form 1 in crosses between Kota and Kanred indicate that the factor for moderate resistance in Kota is also allelomorphous to the factor for immunity in Kanred. Immunity is dominant to moderate resistance and the ratios approximated very closely that of a monohybrid. The factor in Marquis for susceptibility and in Kota for moderate resistance to the same rust form are both allelomorphous to the factor for immunity in Kanred and probably are therefore allelomorphous to each other. Further evidence to this effect was obtained by studying the reaction of the F_3 plants of a cross between Kota and Marquis to this particular physiologic form of rust. These results indicated also that the factors for reaction to form 1 in Kota and Marquis are allelomorphous to each other.

A histological study of certain morphological characteristics of these varieties failed to show structural differences which were correlated with any of the three reaction types and their genetic factors. The basic differences for rust reaction appeared to be primarily physiological. There were wide differences in the proportional amounts of sclerenchyma and collenchyma in the different varieties and hybrids, but there was no close correlation between such structures and their seedling reaction to form 1 in the greenhouse and the reaction to this and other forms in the field.

SUMMARY

1. Kanred winter wheat, when sown in the greenhouse in the fall of the year, shows a continuous growth curve similar to that of spring wheats, with no indication of a period of dormancy. Freezing temperatures are not essential to culm and flower formation.

2. Kanred, when sown in the greenhouse in the late winter or early spring, will remain dormant for several months and not show a continuous growth curve as do the spring wheats. Freezing temperatures in this case act as a necessary stimulus which causes winter wheat to head.

3. There were 4,811 spring types to 442 winter types in the F_2 of the cross between Marquis and Kanred; and 1,177 spring types to 128 winter types in the cross between Kota and Kanred. In the F_3 the ratio approximated 7 lines homozygous for spring habit to 8 heterozygous for spring and winter habit in material in which the winter types had been eliminated in the F_2 . In the F_3 , from a random F_2 , the ratio approximated 7 pure spring to 8 heterozygous to 1 pure winter. Approximately one-half of the heterozygous lines segregated into 15:1 ratios for spring and winter types and the other half into 3:1 ratios.

The results were explained by the hypothesis that Marquis and Kota each contain two pairs of dominant factors for spring habit and Kanred contains the recessive allelomorphs for winter habit.

4. There are several factors concerned with early and late heading in addition to the two factors for growth habit. Earliness was dominant to lateness. The segregation in the F_2 and F_3 was too complex to permit a detailed genetic analysis of the factors concerned with this character. Further studies are necessary before more definite conclusions can be drawn.

5. A backcross of a late spring hybrid selection from Marquis \times Kanred to Marquis failed to produce winter types which typified Kanred in the segregating generations. The segregation for early and late heading suggested at least a two-factor difference with earliness dominant to lateness.

6. Seedling posture shows a general correlation with growth habit. This relationship is only relative and can not be taken as an absolute criterion in the selection of winter types.

7. The immunity of Kanred from several physiologic forms of stem rust in crosses between Marquis and Kanred is governed by a single genetic factor.

8. Immunity is dominant to susceptibility in crosses between Marquis by Kanred and Kota by Kanred to physiologic form 1. The factors for susceptibility to physiologic form 1 in Marquis and for moderate resistance in Kota are both allelomorphic to the factor for immunity in Kanred.

9. A study of F_3 plants from a cross between Marquis and Kota indicated that factors for the reactions of these two varieties to form 1 are also allelomorphic to each other. From these results it was concluded that there is a multiple allelomorphic series of three factors which governs the reaction of Marquis, Kota, and Kanred, to physiologic form 1.

10. No consistent correlations were found between the reaction to form 1 and the structural differences of the parents or the hybrids. The differences in reaction to this form of rust to which Marquis is susceptible and Kota is moderately resistant appear to be due to physiologic causes.

11. The inheritance of growth habit and rust reaction are in accord with Mendelian laws.

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BACTERIAL BLIGHT OF PEA: OVERWINTERING, DISSEMINATION, AND PATHOLOGICAL HISTOLOGY

VLADIMIR SKORIC¹

INTRODUCTION

Bacterial blight of peas was first described by Sackett (5) in Colorado in 1915. It was later noted by Jennison (2) in Montana. Ludwig (4) reported the occurrence of the disease in South Carolina, and it has recently been found by Jones and Linford (3) to be prevalent in pea fields of Wisconsin. From these reports it is clear that the disease is widespread in pea districts, causing more or less severe damage. The symptoms of the disease were described in detail by Sackett and by Ludwig. The possibility of seed transmission was mentioned by Jennison, and a more definite indication that the bacteria overwinter in the form of a dried film on the surface of the seed was given by Jones and Linford. Sackett gives a brief account of the histology of the diseased tissue, in which he mentions the penetration of the organism through wounds and stomata into the stem and leaves and further spread into the underlying parenchyma. According to the findings of the last-mentioned author, the infection does not appear to extend into the pith, sterome, and vascular bundles, although wilting of petioles was not uncommon. He observed a gradual wilting of plants when artificially inoculated, but not a sudden collapse, and therefore he was inclined to consider it a withering rather than a wilting. The present investigation was undertaken in order to determine whether the organism is seed-borne, and to make observations on the method of dissemination. A study was also made of the pathological histology of diseased plants and the ability of the parasite to attack other leguminous plants.

SYMPTOMS OF THE DISEASE

Although Sackett and Ludwig have given detailed accounts of the symptoms of the disease, some additional observations have been made.

When flowers and pods were sprayed with water suspensions of the bacteria, stomatal infection was repeatedly found to occur on the sepals and

¹ This research was carried out at the Department of Plant Pathology, University of Wisconsin, under the direction of Professor L. R. Jones, whom the writer sincerely thanks for helpful criticism and advice. Special acknowledgments are due Professor W. H. Wright for help in cultural work and to Dr. F. R. Jones and Dr. M. B. Linford for helpful suggestions and cooperation during this work.

bracts on the peduncle, the disease later spreading into the stem and pods. The flower-buds were often killed before they could open. When invasion of sepals occurs at the time of pod formation, these soon shrivel and decay. If pods are further developed, the disease spreads to them, causing lesions of various size and cracking of the pods, but the seed is not usually prevented from ripening. When the bacterial invasion on such pods was largely along the dorsal suture (Plate XXIII), the funiculus and surface of some seeds was very often found enveloped in bacterial slime. The funiculus was in this case often deep green and water-soaked. In many instances the seed showed green water-soaked spots only near the one or the other end of the hilum.

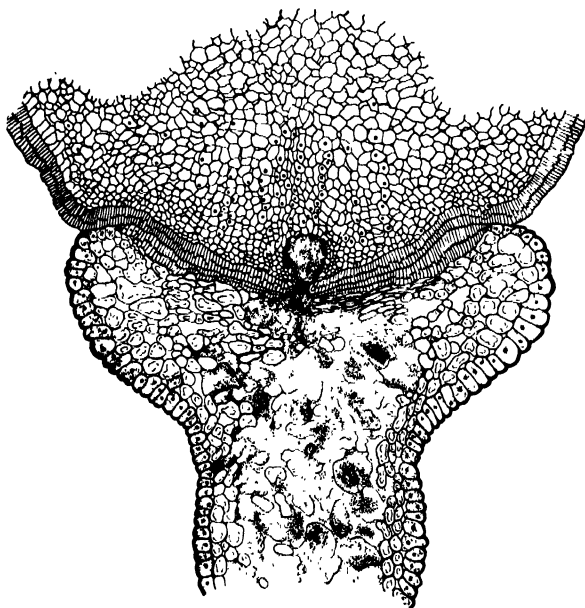


FIG. 1.—Longitudinal section of the funiculus and lower part of the seed showing penetration of bacteria through the micropyle into the seed coat ($\times 105$).

On inoculating the stems of pea plants with needle pricks, the observation was made that many plants so infected showed wilting of leaflets. The larger lesions occurred at the bases of stipules and leaflets, and the whole plant wilted after eight to ten days.

If infected plants were kept in moist chambers for 10 to 20 hours, a grayish-white bacterial ooze in the form of droplets (Plate XXII, B) was observed, which sometimes later became brownish as described by Sackett. Closer observation of infected plants at short intervals revealed the fact that the bacteria extrude from the diseased plant at first in the form of short cirrhi (Plate XXII, A) and only later, by absorption of water, form drop-

lets of slime containing bacteria. Professor L. R. Jones has stated to the writer that he has observed this formation of cirrhi under field conditions to be so abundant on the pod as to simulate the appearance of downy mildew.

ISOLATION AND IDENTIFICATION OF THE CAUSAL ORGANISM

The organism used in these studies was isolated at different times, from living plants and from the surface and inner part of the seed, from the funiculus, and from badly spotted pods, all gathered in July, 1926, at the University Hill Farm near Madison, Wisconsin. Twelve strains from several isolations made during the winter of 1926 and spring of 1927 were grown on the following media: nutrient broth, meat infusion broth, nutrient agar, glucose agar, potato dextrose agar, plain gelatin, glucose gelatin, starch agar, sodium caseinate agar, plain milk agar, plain milk, litmus milk,



FIG. 2.—A detail from figure 1 showing bacteria in micropylar opening and in the cavity in seed coat ($\times 1,000$).

lactose broth, glucose broth, sucrose broth, Buchanan's solution; peptone medium using Difco, Witte's, and Parke & Davis peptone for methyl red and Voges-Proskauer test, Fermi's solution, Uschinsky's solution with corrected reaction to pH 7.0 and with uncorrected reaction, and finally in Cohn's solution.

The behavior of all the strains of the organism on culture media, which were identical with those used by Sackett, was in all cases the same excepting slight differences in plain and litmus milk, where soft curd production was not observed. The cultivation of the organism on starch agar has given in all twelve strains distinct hydrolysis of starch below, and to a certain

extent around, the giant-colonies. The organism grew very abundantly and rapidly in Buchanan's solution and in different peptone media, giving a negative test in both methyl red and Voges-Proskauer test. In spite of the insignificant cultural differences in plain and litmus milk, and in spite of the hydrolysis of starch—which may have been due to the differences in the method used by Sackett and that used in this investigation, we consider that the organism with which we have been working is identical with *Pseudomonas pisi* Sackett (*Phytomonas pisi* (Sackett) S. A. B.).

OVERWINTERING AND SEED TRANSMISSION OF THE ORGANISM

As has been mentioned previously, there are suggestions in the literature that the organism may be seed-borne. To solve this problem, trials were made to isolate the organism from the seed. The seed used for this purpose were in closed pods which had been carefully collected in June, 1926. The pods showed abundant bacterial lesions and were therefore considered very suitable for the isolation of the organism from the seed. The pods were air-dried and stored in the laboratory until November, when the first attempts to isolate the organism were made.

The surface of the pods which were found to be completely closed was disinfected by treatment for five minutes in mercuric chloride and washed in sterile water. Then the pod was opened under aseptic conditions in a sterile petri dish. The seeds were transferred with sterile forceps to sterile water blanks, which were shaken thoroughly from time to time and allowed to stand for half an hour to permit the distribution of bacteria in the water. The plates poured from the water used in these first attempts remained sterile. Since it was suspected that the time in water was too short to allow the distribution of the bacteria, in the next isolations the seed were allowed to stand in sterile water blanks over night and plates were poured from this suspension. This time numerous colonies were obtained on potato dextrose agar quite like the colonies of *Pseudomonas pisi*, and later inoculations have shown that the organism thus obtained is pathogenic to pea plants, producing characteristic lesions on the stems and abundant stomatal infection on stipules and leaves.

Shortly thereafter, it was found by closer examination of the seed from infected pods that white, dry films are present on their surfaces. When seed with such films were used for isolation, the development of the organism on plates was very abundant, and in most cases a pure culture of *Pseudomonas pisi* was obtained. To establish whether this film contains bacteria, a small piece of the film was transferred to a sterile water drop on a sterile slide, crushed, and allowed to spread and dry. After fixing and staining with carbol fuchsin, it was possible to see that the whole microscopic field

contained shortened rods, sometimes almost cocci-like, embedded in slime. To prove that the bacteria contained in the dry film were alive and identical with the causal organism of bacterial blight, an attempt was made to plate out a small part of the bacterial film. About 3 square millimeters of film were taken with a sterile scalpel, transferred, and crushed in a water drop in a sterile petri dish. After a short time, when it was considered that the organism was properly distributed in the drop, one loopful was transferred to each of a second and third petri dish. Plates were poured with potato dextrose agar, and after 36 hours bacteria developed very abundantly in the first plate, a few colonies in the second, and none in the third. That these were pure cultures of the pea blight organism was shown by examination and confirmed by the production of infection on inoculated plants. Further observations have shown that, although the film may be invisible to the naked eye, its presence can be demonstrated by isolation of the organism from the

TABLE 1.—*The number of bacteria present on the surface of seed from pea pods infected with bacterial blight*

Seed no.	Isolation date, 1927	No. of dilution plates	Average no. of colonies ^a	Inoculation results ^b	Check
1	Jan. 5	3	3540	+	Healthy
2	do	3	0		
3	do	3	94	+	Healthy
4	do	3	20	+	do
5	Feb. 8	3	30	+	do
6	do	3	0		
7	Mar. 2	5	15400	+	Healthy
8	do	3	0		
9	do	3	380	+	Healthy
10	Apr. 6	3	70	+	do
11	do	3	623	+	do
12	do	3	0		
13	May 12	4	1840	+	Healthy
14	do	3	0		
15	June 2	5	yellow saprophyte		
16	do	5	do		
17	do	3	0		
18	do	5	21200	+	Healthy
19	do	3	1500	+	do
20	do	3	40	+	do
21	do	3	0		

^a In the cases where the number of the colonies was large, a conspicuous bacterial film was always present.

^b + = Abundant infection.

surface of the seed. Several attempts were made to determine the number of viable bacteria on the surface of seed. The seed were placed in sterile water blanks and kept over night at 4° C. to permit the diffusion of the organism in the water, the temperature being low enough to prevent their multiplication. According to the cloudiness of the suspension, three to five dilution plates were poured, the colonies counted, and the pathogenicity established by inoculations. In table 1 are given averages of plate-counts showing the number of viable bacteria on individual seed. The results are variable, as would be expected, considering the differences in the severity of pod infection.

In several cases it was noted that seed having no visible trace of bacterial film on the surface nevertheless gave rise to milky clouds when the water blanks in which they had been soaked for some time were shaken. This indicated that there might be a possibility of the overwintering of the organism other than on the surface of the seed. When such seed were thoroughly

TABLE 2.—*Isolations of Pseudomonas pisi from pea seed over a period of 10 months after collection*

Date of isolation	No. of seed examined	No. of seed containing organism	Manner of inoculation	Result of inoculation ^a	Remarks
Nov. 10, 1926	10	6	Needle-pricks and spraying with bacterial suspension	+	Abundant stomatal infection and stem lesions
Dec. 9, 1926	10	4	do	+	do
Jan. 3, 1927	8	3	do	+	Stem, pods, sepals, and leaves abundantly infected
Feb. 5, 1927	8	4	Sprayed with bacterial suspension	+	Stomatal infection on sepals and leaves
Mar. 5, 1927	2	2	do	+	Seeds with water-soaked spots
Apr. 6, 1927	4	3	do	+	do
May 12, 1927	5	3	do	+	Two seeds with water-soaked spots
June 2, 1927	10	6	do	+	Three seeds with water-soaked spots

^a + = abundant infection.

examined, a water-soaked spot beside the hilum was discovered (Plate XXI, A). Water-soaked lesions completely surrounding the hilum were seldom found.

To test whether this discoloration is a local change in color so often found on dry pea seed or a water-soaking caused by the penetration of the organism, some seed showing these symptoms were disinfected for five minutes in mercuric chloride and washed in sterile water. The seed were allowed to stand overnight in sterile water and plates were poured from this water. Positive inoculations with the organism isolated from such seed confirmed its identity with *Pseudomonas pisi*. The isolations were repeated several times with the same success.

The abundance of the organism in a dormant condition, but alive and virulent on the surface and within the seed during a period of 10 months is shown in table 2.

The percentage of seed with clearly visible films and with water-soaked spots in several lots taken from heavily infected pods was determined. The average of counts gave 13 per cent of seed with the bacterial film on the surface and 2.5 per cent with lesions.

The next step was to establish whether the disease can be transmitted by infected seed to the young plant. For this purpose, seed which showed water-soaked spots and which came from underneath lesions on the pods were selected. The seed bearing water-soaked spots were considered to be internally infected, and those from below pod lesions to be carrying the organism on the surface. The seed with internal infection were disinfected with mercuric chloride for five minutes, and the seed with the organism on the surface were not treated. As a control, seed from healthy pods of the same variety were sown in sterile soil and pots. The result of two sowings are shown in table 3.

TABLE 3.—*The development of blighted pea plants from infected seed and the effect of soil moisture on the development*

Date of sowing, 1927	Pot no.	No. seed	Location of organism	Treated with	No. of diseased plants	Remarks
Feb. 1	1-6	33	In seed	HgCl ₂	6	Abundantly watered
do	7-29	136	On seed	9	do
do	30-34	40	Control	0	do
Feb. 28	1-3	18	In seed	HgCl ₂	0	Scantly watered
do	4-6	16	On seed	0	do
do	7-8	16	Control	0	do

The sowing of infected seed has shown that the disease can be transmitted by this means, but at the same time the importance of the water content of the soil in the transmission of the disease has been indicated.

The appearance of the symptoms on diseased plants was very interesting, suggesting that the organism usually does not penetrate into the cotyledons and embryo in the seed, but that penetration takes place through the surface of the developing seedling. Most of the diseased plants, developed from infected seed, showed the infection only on the lowest pair of stipules. Some of them showed symptoms on the first, second, and third stipules, and first and second pair of leaflets. All organs of a few plants became infected, especially the apices, whence the disease spread downward and killed the

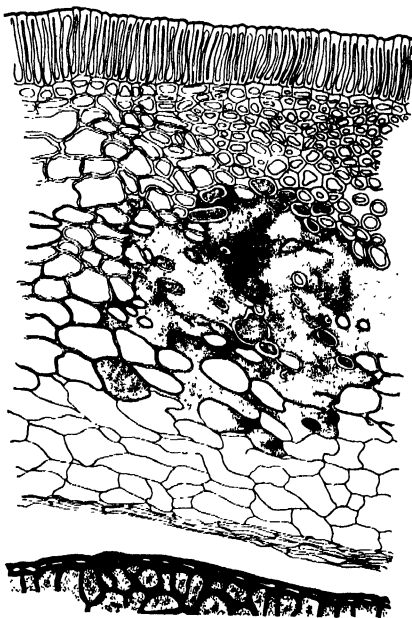


FIG. 3.—Cross-section of seed coat of ripe seed showing the location of bacteria ($\times 460$).

young plants. After the development of symptoms on such plants, it was possible to find that the position of many of the lesions corresponded to that of the lesions on the stipules which were covering them during the emergence of the plant from the soil. This distribution of lesions indicates that the outer covering of the plumule becomes infected by touching the infected seed coat and transmits the disease to some or all the organs beneath it according to the rapidity or slowness of the development of the young plant. This is a similar method of infection to that described by Jones and Linford (3) in *Ascochyta pisi*.

The severe outbreaks of the disease in pea fields in previous years have suggested to several observers that the organism may survive in the soil.

A few attempts by the writer to determine whether or not the organism was present in soil on which diseased peas had been found during several previous years have given negative results.

FIELD OBSERVATIONS ON INCIDENCE AND DISSEMINATION OF THE DISEASE

As it had been shown that the organism is viable on and in the seed, and that from such seed diseased plants developed in the greenhouse, a study was also made of the development of the disease under field conditions. For this purpose several pea fields near Columbus, Wisconsin, were visited, and especial consideration was given to trial plots of E. J. Renard at the University Hill Farm near Madison, Wisconsin. When the pea plants were still quite small (about 10 to 12 cm. high), it was not difficult to distinguish plants which showed the disease in a form characteristic for plants infected

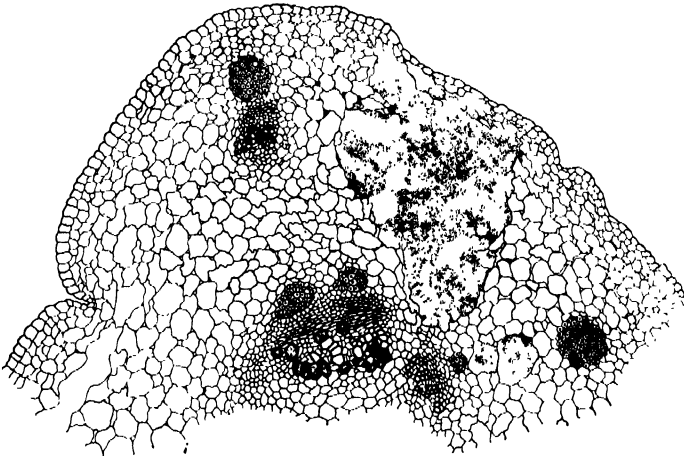


FIG. 4.—Cross-section of a part of the stem with large bacterial cavity in cortex and vascular invasion of leaf-trace and of vascular bundle ($\times 105$).

from seed (Plate XXI, B). The lower stipules and leaves were at this time more or less covered with old lesions of bacterial blight, and there was also secondary infection in the upper part of the plants strikingly like the symptoms of diseased plants which developed from infected seed in the greenhouse experiments. Around such plants were found several plants showing only secondary infection. These plants with secondary infection were found in most cases in the same drill-row and sometimes in the next row when the rows were close together. In the fields where the plants were older, the origin of the disease from one infection center was not so evident. Especially instructive in this regard were the trial plots of the Leonard Seed Company at Columbus, Wis., where Alaska peas from different sources were planted in a field which had not grown peas before. Among many rows

sown at a distance of 75 centimeters apart, each containing about a hundred plants, there were found only three plants apparently infected from the seed and situated in three different rows. Here again the plants around these infected individuals showed only secondary infection. Fortunately, 400 seed remained of the sample which had been used to plant one of the plots in which a diseased plant was found. Among these 400 seed two were found with water-soaked lesions, further evidence that the diseased plants developed from infected seed.

The trial plots at the University Hill Farm also have confirmed previously mentioned findings. All the plots were examined thoroughly for blighted plants: many of them did not show any primary-infected plants; others showed one to nine of such plants in a thousand. Both diseased and

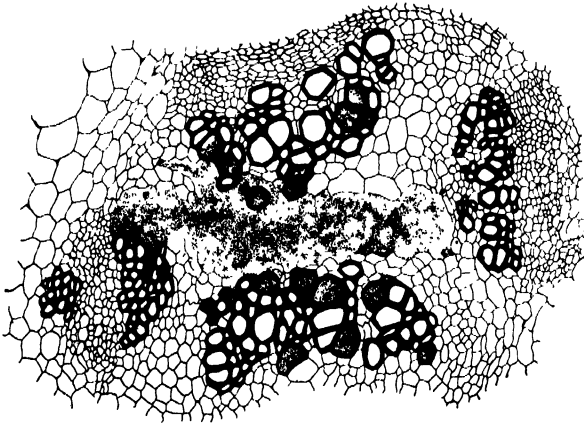


FIG. 5.—Cross-section of central part of the stem with bacterial cavity in the pith and invaded vascular bundles ($\times 460$).

healthy plants were grown from seeds from almost every state represented in the trial. Individual examinations of plants in this pea field have shown that in many rows there were plants showing a small amount of secondary infection, although they were not near primary-infected plants. It was noticed that such plants occurred mostly in the lower parts of the field. These infections may be explained by the distribution of the organism in drainage water, as has been shown by Carsner (1) to be the case with angular-leaf spot of cucumber.

PATHOLOGICAL HISTOLOGY

The regular occurrence of water-soaked lesions on one or the other side of the hilum, and nowhere else on the seed surface, suggested that there must be a definite method of migration of the organism into the seed. To trace this migration, pod inoculations were made in two ways: by spraying

with water suspensions of the bacterium; and by introducing the organism by needle pricks into the cavity of the pod, taking care to avoid wounding the seed. Both methods have led to production of water-soaked lesions on the seeds, but again only near the hilum. At the same time more or less extended water-soaking of the funiculus was observed. Young seed with heavily infected funiculi did not develop further; many of them collapsed and dried up. Infected pods and seed were fixed in formal-acetic alcohol, imbedded, and sectioned in the usual way. Sections were stained in weak safranin or carbol fuchsin, Rose Bengal (counter-stained with light green in clove oil), or Giemsa stain, and counter-stained with two per cent safranin in alcohol. The sections show that the tissue of the infected pods was largely invaded by the bacteria, which were abundant in the cells and interstitial spaces. Bacteria penetrated through the wall of the pod, accumulating on the inner surface, on the funiculus, and on the seed, without being able to penetrate into the seed or funiculus. When the bacteria occupied the larger part of the tissue beneath the dorsal suture, they penetrated into the tissue of the funiculus and migrated toward the seed. In some cases the bacteria destroyed so much of the tissue of the lower part of the funiculus that the seed underwent no further development. In other cases the organism traversed the funiculus to the micropylar opening, and through this natural opening entered the integuments (Figs. 1 and 2), preventing the development of the young seed. If the seed was sufficiently developed when the bacteria reached the micropylar opening, they entered into the seed coat and the seed ripened normally. In such cases, if the bacteria penetrated in large numbers, water-soaking near the hilum resulted, but a slight penetration may occur without any macroscopically visible signs on the surface of the seed. Sections were made of mature seed collected in 1926 and also of seed nearly mature with water-soaked lesions from artificial inoculations in the greenhouse. Also seed from field material collected in June, 1926, was examined for the same purpose. In both cases, it was possible to demonstrate that the bacteria are confined to the seed coat. They were either intercellular or intracellular in position and produced smaller or larger cavities filled with bacteria embedded in slime (Fig. 3). It was not possible to observe whether the bacteria penetrated the cotyledons, but in some instances they were found occupying the cells of the seed coat in close proximity to the rootlet of the embryo. Therefore it seems probable that sometimes they may enter the young embryo. Such infection may explain that fact that the more heavily infected seed fails to develop at all or dies soon after producing only a small rootlet. The organism has been isolated from seed and seedlings killed in this manner.

Artificial inoculations of the stem by needle pricks have in many instances produced wilting of leaflets and of whole plants, but some plants

which have developed from infected seed also wilted. Therefore it was assumed that the bacteria entered the vascular bundles and caused the wilting by plugging the vessels. The examination of slides obtained from such wilted plants has shown the vessels filled with bacteria (Figs. 6 and 7). Since all the vessels are not completely plugged with bacteria, it is probable that the abundant slime produced by them is playing an important rôle in preventing the passage of water to the higher parts of the plant. High absorptive power of this slime would explain how the organism may enter comparatively firm vessels. Somewhat below the epidermal layer of the pea stem there occur normally four cavities which permit a very rapid and abundant development of bacteria and bacterial slime. The high pressure

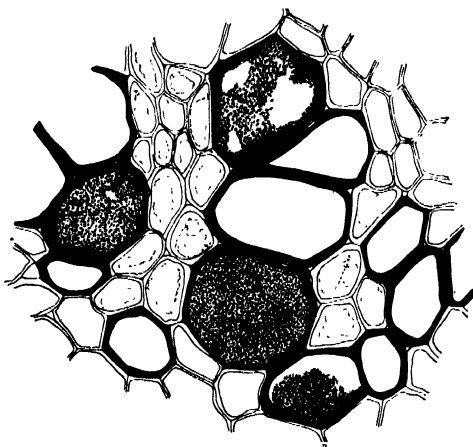


FIG. 6.—Cross-section of vessels occupied by bacteria ($\times 1,000$).

produced by the slime tears the surrounding tissue, producing large cavities (Fig. 4). In the surrounding parenchyma the organism can be found both intercellular and intracellular, enlarging the occupied area in the manner mentioned. In longitudinal sections of the stem the same process can be seen near the vascular bundles, and the pushing activity of slime and bacteria is visible in lens-shaped cavities which contain ruptured vessels either in or at their margin. However, this does not seem to be the only method of penetration and spreading of the organism in the plant, because in some instances it was possible to find that the bacteria dissolved the wall between two vessels, and penetrated from one into the other. The large cavities produced by the organism in the parenchyma of the pith (Fig. 5) and cortex can not be entirely accounted for by rupturing and crushing of tissue. Some parts of the cavity at least appear to result from solvent action of the organism.

Although under field conditions the organism is largely a parenchyma invader, it sometimes may enter the vessels. It is of special interest to

point out that where the stipules and leaves show large lesions at their bases, the vessels of the leaves and leaf traces and sometimes the vascular bundles of the stem are occupied by bacteria. This was found to be true not only in greenhouse experiments but also in the material collected in the field.

As has been stated by Sackett and later confirmed by Ludwig, the organism very readily enters the plant through wounds. Sackett has shown that stomatal penetration into the leaves may also take place. Experiments performed during the present investigations have confirmed the statements of the above-mentioned workers. We have succeeded in securing abundant stomatal infection on leaves (Fig. 8), but only in very few instances does it appear that stomatal penetration has taken place on the stem and pods.

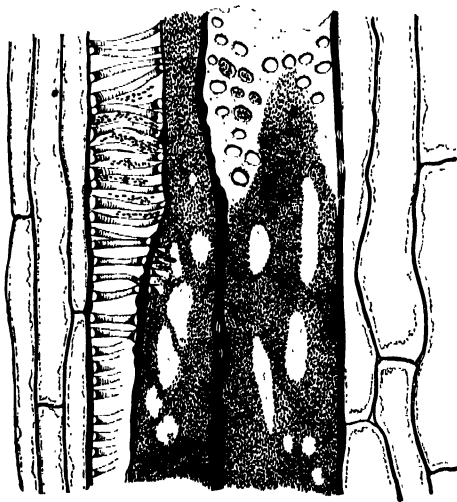


FIG. 7.—Longitudinal section of vessels occupied by bacteria ($\times 1,000$).

Even when the plants were kept for 48 hours in a moist chamber before inoculation to favor the penetration of the organism through stomata into pods and stems, negative results were obtained. It is worth mentioning that the staining reactions of the guard-cells in pods show differences in comparison with those in leaves, but it is not possible at present to determine their nature.

HOST RANGE

Sackett has shown that the organism attacks field and garden peas, and that it does not attack alfalfa, sweet clover, crimson clover, mammoth clover, cow peas, and garden beans. We have inoculated many other species in addition to those tried by Sackett, as shown in table 4. These pathogenicity tests have partly confirmed earlier findings by Sackett, but they have shown

at the same time that there are other leguminous plants susceptible to the organism. *Lathyrus odoratus* and *L. latifolius* showed symptoms quite similar to those in garden and field peas, with the difference that, although abundant stomatal infection was produced, the lesions were comparatively small. *Dolichos lablab* showed slight stomatal invasion and long black streaks along the stems and petioles. These streaks under microscopic examination have shown that the organism occurs principally in some of the vascular bundles, the surrounding parenchyma being invaded only to a small extent. The plant reacted to this attack by plugging of the vessels

TABLE 4.—The results of inoculating various leguminous plants with *Pseudomonas pisi*

Plant tested	No. plants inoculated	No. of checks	Result of inoculation ^a	Result of reiso- lation and rein- oculation into pea plant ^a
<i>Pueraria thunbergiana</i> . . .	3	1	—	
<i>Phaseolus aconitifolius</i> . . .	4	1	—	
<i>P. angularis</i>	2	1	—	
<i>P. vulgaris</i>	6	2	—	
<i>Melilotus officinalis</i>	5	1	—	
<i>Medicago sativa</i>	8	5	—	
<i>M. lupulina</i>	6	3	—	
<i>Trifolium incarnatum</i>	2	1	—	
<i>T. pratense</i>	2	1	—	
<i>Cicer arietinum</i>	3	1	—	
<i>Lens esculenta</i>	4	1	—	
<i>Lespedeza bicolor</i>	5	1	—	
<i>Arachys hypogaea</i>	5	3	—	
<i>Lupinus luteus</i>	2	1	—	
<i>L. albus</i>	3	1	—	
<i>Vicia faba</i>	5	1	—	
<i>V. gigantea</i>	15	6	—	
<i>V. villosa</i>	12	5	—	
<i>V. sativa</i>	10	2	—	
<i>V. pannonica</i>	14	4	—	
<i>Dolichos lablab</i>	9	3	+	+
<i>Lathyrus odoratus</i>	14	6	+	+
<i>L. latifolius</i>	6	2	+	+
<i>Lathyrus</i> , wild sp. from Utah	5	2	—	
Progressus, white cow pea	4	1	+	+
Early black cow pea	5	1	+	+
New Erg. cow pea	5	1	+	+
Holly Brook soy bean	3	1	—	
Wisconsin black soy bean	3	1	—	
<i>Pisum sativum</i>	14	5	+	+

^a + = Infection. — = No infection.

and abundant cell divisions around the invaded vascular bundles. The disease developed on cow peas in a similar manner as in *Dolichos lablab* except that there was no stomatal penetration. The fact that *D. lablab* is a host plant for bacterial blight of bean (*Pseudomonas phaseoli*) suggested that it might be possible to infect beans with *Pseudomonas pisi* reisolated from this host. Repeated trials to inoculate beans with strains of *P. pisi* obtained from *D. lablab* have failed to produce the disease.

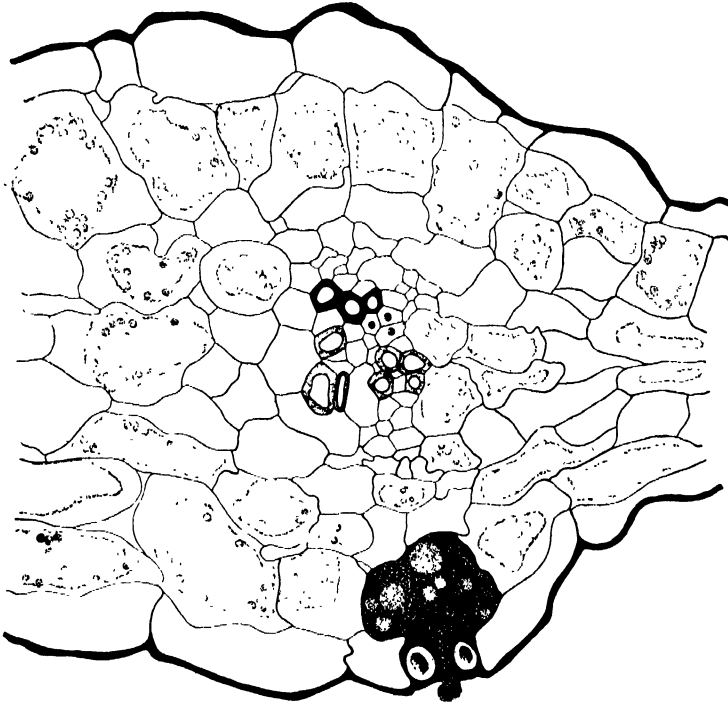


FIG. 8.—Cross-section of a part of leaf showing stomatal penetration by *Pseudomonas pisi* ($\times 1,000$).

The cultures of the organism used in inoculating other leguminous plants were at the same time used in inoculating peas and in every case produced the disease. In all cases where the disease appeared on inoculated leguminous plants, the organism was reisolated and produced infection on inoculated pea plants. Furthermore, on culture media, the organism reisolated from these plants has shown its identity with bacterial blight of pea.

CONTROL

The proof that pea blight is seed borne, and evidences of its transmission in this way to young plants both in the greenhouse and under field conditions, should aid in elaborating control measures. More attention should

be given to the pea fields from which the seed is to be selected for the subsequent year. The fact that the organism overwinters on the surface of the seed and within the seed coat would appear to necessitate disinfection of seed with substances which have a lasting bactericidal value. Such substances would kill not only the bacteria on the surface of the seed, but also would prevent the infection of the young seedlings by the organism which from time to time diffuses from the seed coat. Extensive tests with different disinfecting agents should be made.

SUMMARY

Infection by *Pseudomonas pisi*, the causal organism of bacterial blight of pea, often takes place on sepals, spreading towards the peduncle and pods, killing flowers, and causing shrivelling of young pods. Badly infected pods contain seed which often have more or less extensive bacterial films on the surfaces, and sometimes show a water-soaked spot near the hilum.

During moist weather, bacterial ooze is present. The bacteria exude from the infected plant at first in the form of short cirrhi, which later absorb water, forming droplets of slime containing bacteria.

The organism overwinters in the form of a dry bacterial film on the surface of the seed and also in the seed coat. It remains alive at least for ten months. When infected seed are sown, a certain percentage of plants coming from them will be infected, and such infected plants are centers for the dissemination of the disease in the field.

The organism penetrates through wounds, but stomatal infection on leaves and sepals occurs abundantly. Spreading through intercellular spaces, it enters readily into the parenchyma cells not only of the cortex, but also of the pith. In case of favorable conditions for the disease, the bacteria produce large cavities in the plant, breaking down cell walls by high pressure of bacterial slime and by chemical action. The important effect of this action is the breaking of vessels and entrance of the organism into the vascular bundles, with consequent wilting of leaflets and occasionally of the whole plant.

The entrance of the bacteria into the pod seems to be largely through wounds. Spreading between and through the cells, they penetrate through the pod wall and form abundant bacterial slime on the inner side of the pod and on the surface of the seed. The migration of the organism into the seed comes about only by passage from invaded pod tissue into the funiculus, and from there through the micropyle into the seed coat, where it remains alive in a dormant condition.

The organism is pathogenic not only to field and garden peas but to hyacinth bean (*Dolichos lablab*), cow peas (*Vigna sp.*), sweet pea (*Lathyrus*

odoratus), everlasting pea (*Lathyrus latifolius*), and probably to some other species of *Lathyrus*.

Seed treatment by suitable disinfectants would seem to offer possibilities for the control of the disease.

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4. LUDWIG, C. A. *Pseudomonas (Phytomonas) pisi* Sackett, the cause of a pod spot of garden peas. Phytopath. 16: 177. 1926.
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EXPLANATION OF PLATES

PLATE XXI

- A. Ripe pea seeds with water-soaked lesions near the hila.
- B. Symptoms of bacterial blight on pea plant grown from infected seed.

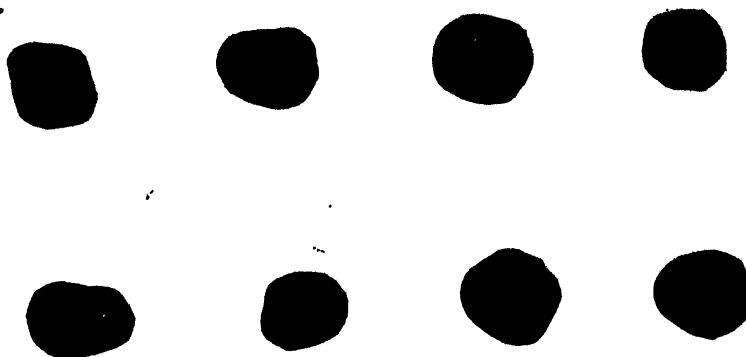
PLATE XXII

- A. Pea plant in the foreground showing bacterial cirrhi on the stem.
- B. Pea plants showing bacterial ooze.

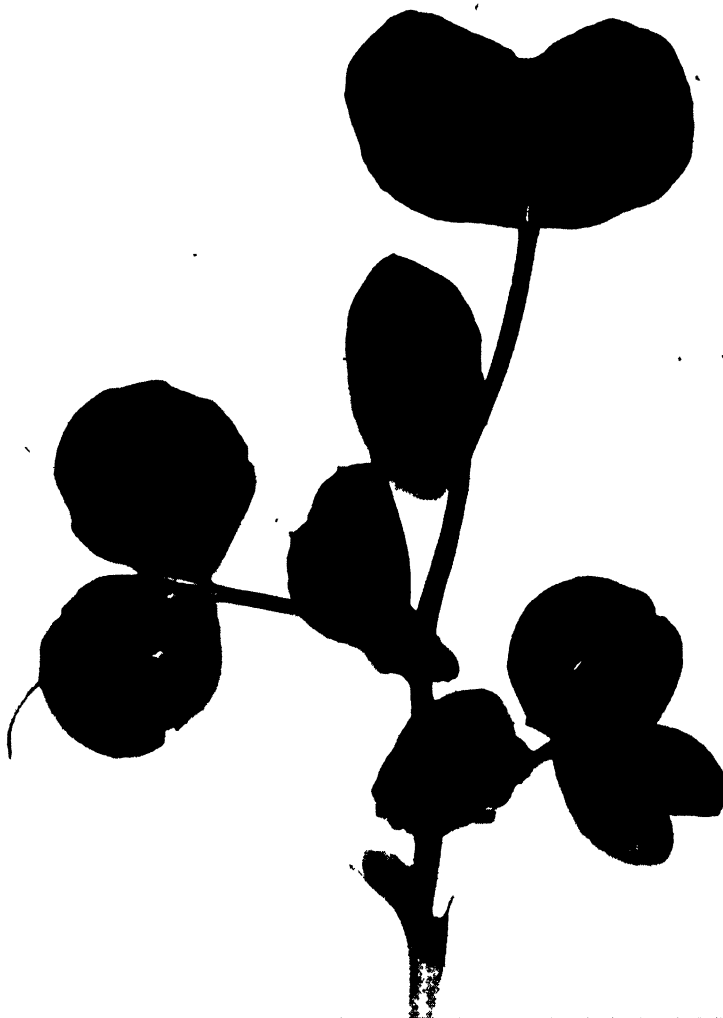
PLATE XXIII

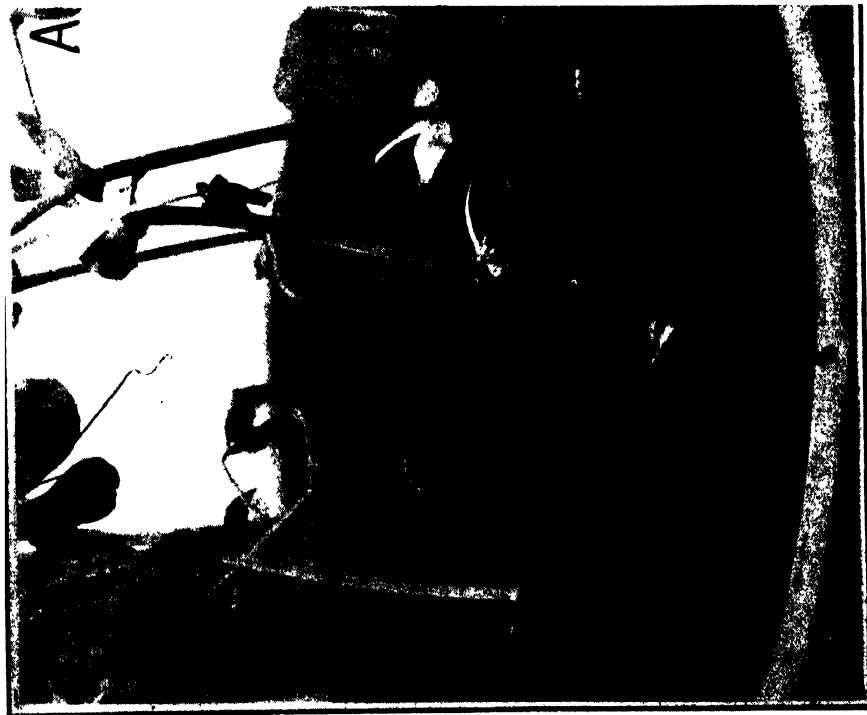
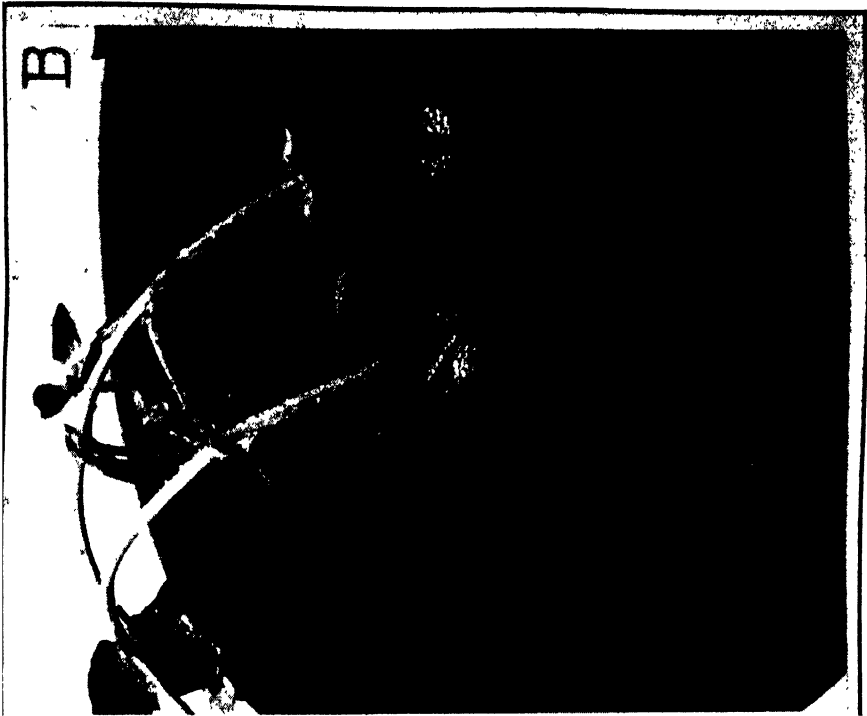
Pea plant showing the infection on pods and the peduncle.

A.



B.







DWARF OF BLACKBERRIES¹

S. M. ZELLER

INTRODUCTION

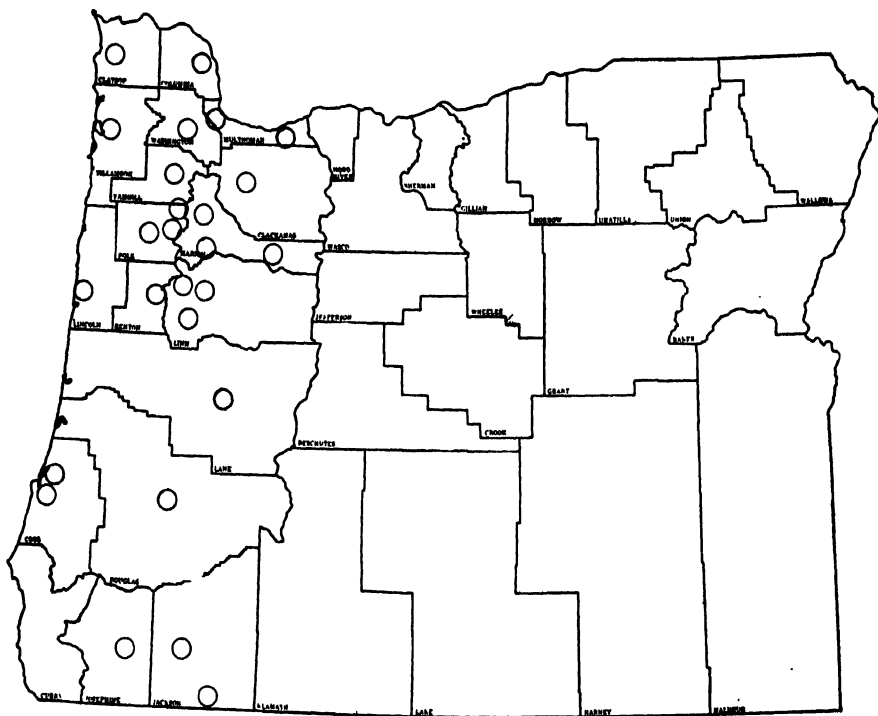
A dwarfing disease of the vining type of blackberries represented by the Logan and Phenomenal varieties has been known to exist in the Pacific Coast states of California, Oregon, and Washington since 1918. In that year Darrow (2) mentioned in a discussion of the Phenomenal blackberry that "in many sections, because of a disease which results in a dwarfed growth of the plants, only two or three crops can be harvested before the plantation becomes unprofitable. . . . Compared with the loganberry the plants are more subject to the dwarfing disease and are shorter lived." Although dwarf has been known on the Coast for some years, it is only recently that the disease has received detailed attention by the writer. His observations of the symptoms and nature of the disease and methods for its control are recorded in this paper.

DISTRIBUTION AND OCCURRENCE

The geographic distribution of the dwarf disease is limited so far as known to various localities in California where Logan and Phenomenal berries are grown, to the coastal slope, Willamette and Umpqua valleys in Oregon, and the portion of Washington west of the Cascade Mountains. The disease has been reported from practically every region of these coastal states in which Phenomenal blackberries are grown. In fact the occurrence of the disease seems to be associated definitely with the Phenomenal berry but usually spreads to the Loganberry when the two varieties are planted in close proximity. The accompanying map of Oregon (Fig. 1) shows the stations where dwarf has been observed or from which specimens have been sent to the writer for identification.

The studies on dwarf by the writer have been conducted in the main in Oregon, and so the consideration of the occurrence here given is based principally on Oregon conditions. In some plantings affected by the disease the damage is slight or relatively unimportant, while in others the losses of plants vary from serious to total. The losses have been so serious in some Loganberry plantings that the entire acreages have been grubbed out, while 100 per cent of the plants were found diseased in one planting containing

¹ Published by permission of the Director of the Oregon Agricultural Experiment Station.



600 Phenomenal plants. In one case near Corvallis there were several diseased plants in one row of Phenomenals along one side of a three-acre planting of Loganberries. The disease gradually spread into the Loganberries until all of the Phenomenals and about 50 per cent of the rows of Loganberries adjacent to them have been removed, but still there are scattering plants in the remaining rows which manifest symptoms of dwarf. At any one time during the last four seasons the highest count of diseased plants in this case was 6 per cent. In a three-year old planting of Loganberries in Lane County 19 per cent of diseased plants were found. The owner stated that the diseased plants had increased in number for three years. The tips for the planting had been purchased from a grower who had raised both Logan and Phenomenal berries. Many other similar cases might be cited, and many cases of very low percentages of diseased plants have been found. Few plantings of the Phenomenal blackberry have been found without dwarf, but Loganberries planted alone seldom have the disease. Dwarf has not been found in Loganberries except in cases where its origin could be traced directly to the Phenomenal or to its probable transmission from it.



FIG. 2. The first noticeable symptoms of dwarf in a Loganberry plant infected after it was mature. The canes are spindly with small leaves which usually have a normal color. The healthy cane is thrown over the top for the sake of comparison.

ECONOMIC IMPORTANCE OF DWARF

The economic importance of the dwarf disease is measured essentially by the percentage of plants affected. A plant which has had dwarf for one full season is valueless. Therefore the economic losses are really shown in the examples mentioned above. Taken as a whole, the loss through dwarf to the Loganberry industry in the three Pacific Coast states is very slight, but many individual growers have been found to have a high enough percentage of infected plants to make the remainder of healthy plants fall far below a financially successful planting, making total eradication necessary.

HOST PLANTS

The Phenomenal and Loganberry are both susceptible to dwarf, the disease being practically limited to these two berries. The Phenomenal is by far the more susceptible. With the exception of a few plants each of the

Kittitany and Cory's Thornless blackberries, no other hosts for this disease have been observed. Five Kittitany plants in one planting and three plants of Cory's Thornless in another single planting were found to have symptoms

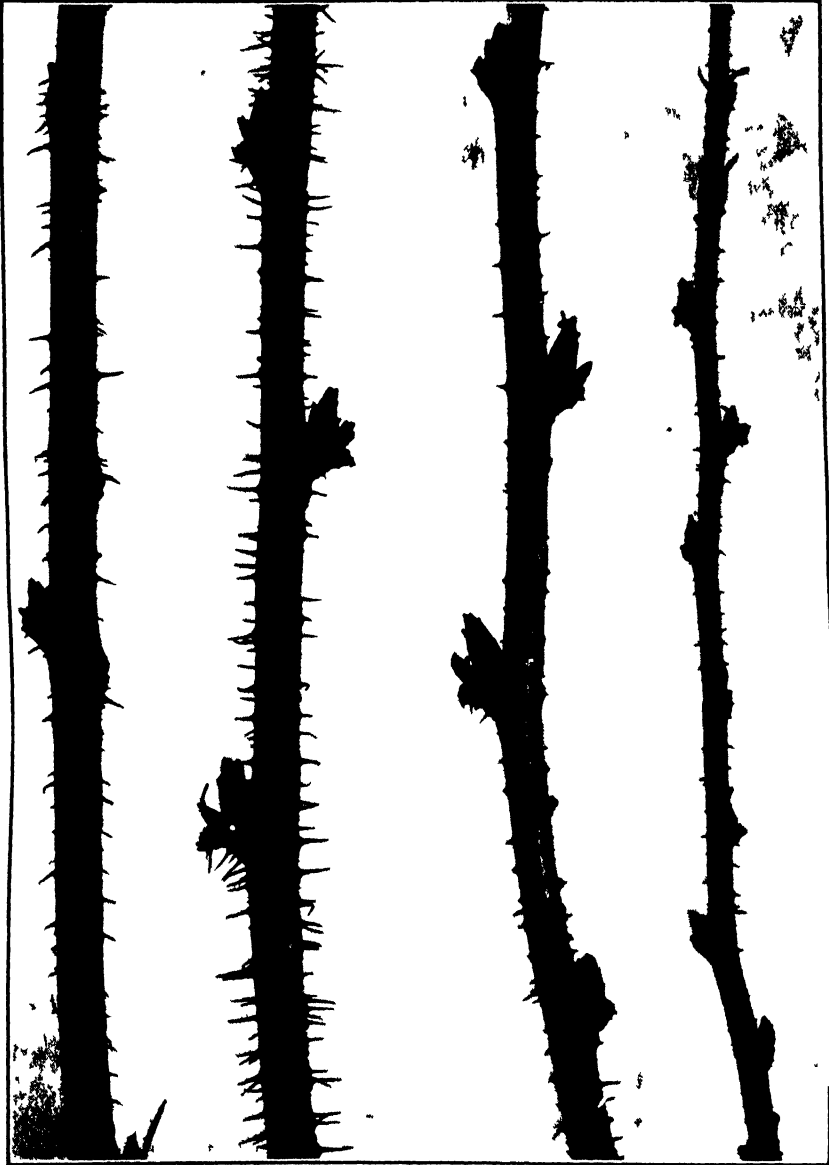


FIG. 3. Portion of a healthy Loganberry cane (left) and portions of three canes (right) from dwarfed plants. Notice the shortened internodes of the diseased canes. There are usually more than one bud at a node on the diseased canes and one bud at a node on the healthy canes

similar to dwarf, and, although transmission experiments have not definitely proved the disease in these two varieties to be dwarf, the symptoms and behavior of the plants are the same.

SYMPTOMS OF DWARF

General Symptoms.—The progress of the dwarf disease in the plant is characterized by distinct morphological and physiological changes, most marked in the leaves and stems, and to a certain extent in the fruit. These consist principally of an abnormal color accompanied by certain malformations and a general dwarfing. The symptoms vary somewhat according to the age of the plants at the time of infection, but the general characters are

..... es of growth after the initial year of infection.



FIG. 4. Portion of a severely dwarfed cane of the Phenomenal blackberry taken just after the leaves had started in the spring. Notice the extremely short internodes and the great number of buds at some nodes.

Symptoms on Mature Plants Infected in the Field.—The first apparent manifestation of the disease in a mature plant is not so much a dwarfing of the stems as of the leaves. Instead of a foreshortening of the stems as may be apparent in subsequent years, the canes during the first year of noticeable symptoms are spindling and flexuous. The leaves are much smaller than leaves of healthy plants, and the leaflets are of abnormal shape—obovate instead of ovate as in normal leaves. At this stage the leaves are of a normal color and the whole plant presents an appearance as illustrated in figure 2.

In the fall of this first year there are several small buds, usually three or more, set at each node instead of the usual single bud in healthy canes. In the winter condition this characteristic of the buds presents quite an apparent contrast to those of healthy canes (Fig. 3). The next spring all of the buds at each node grow (Fig. 4) and produce short laterals, but one finally becomes predominant and the others dry up when they are about one or two inches long. The laterals produce flowering buds rather normally and a meager amount of fruit. After the first leaves are out this second



FIG. 5. Current season symptoms on a Loganberry plant which was infected the first year from the young tip. Notice the peculiar crinkling of the leaves. This plant was caged May 11, 1925, viruliferous aphids were introduced May 14, and on May 20 there was first noticed slight necrosis along the mesophyll between the lateral veins. First dwarf symptoms were noticed on June 6. Photographed July 17.

spring the canes take on a rosetted appearance due to the crowding of the leaves at the nodes. The new canes which are produced the second season are typical of dwarf plants in all of their later history (Figs. 10 and 11).

Symptoms on Plants Infected in the Young Tips.—The first symptom in all cases is necrosis, which appears in the young leaves upon which the viruliferous insects have fed. This necrosis may be slight or severe, as described later in this paper. Leaves which appear on all parts of the young plants after inception of the disease have a peculiar distortion and crinkliness



FIG. 6. This young Loganberry tip was caged May 11, 1925, and non-viruliferous aphids were introduced on May 14. This plant has remained healthy.

(Figs. 5 and 7) and are much smaller than on normal plants of the same age (Figs. 6 and 12). These leaf symptoms characterize a majority of the leaves produced during the remainder of the first season. The canes grow spindly and flexuous. The second season the plants (Fig. 8) take on the appearance of diseased mature plants in their first season, as described above, becoming typical dwarf plants the third season. The detailed symptoms following infection by insects will be discussed later in this paper in connection with transmission experiments.

Leaf Symptoms.—The pattern of coloring, shapes and distortions which the leaves of dwarf plants assume vary with the conditions under which the leaves are produced, or during the current year of infection the leaf symptoms vary with the age of the plant at the time of infection. The stage of development of individual leaves of course influences the appearance of the



FIG. 7. An individual tip from the plant shown in figure 5, illustrating the mottled and crinkled or savoyed condition of the leaflets.

symptoms, for leaves produced during succulent growth are different in texture than those produced during more xerophytic growth.

Just after disease inception in young plants which are in their first year from tips, the leaf symptoms are as shown in figure 7. Previous to this condition there is necrosis, which will be described later in this paper. Following the necrosis all growth of the current season produces the crinkled leaves. It appears from the crinkling that the veins of the leaves grow less rapidly than the mesophyll, so that a more or less bullated condition follows the spaces between the primary veins. In many of such leaves the margins are extremely irregular. The leaflets become more orbicular and broadly ovate as the season progresses and even become obovate, in contrast to the graceful ovate leaflets of healthy plants. The leaves are not usually abnormal in color during the current season of infection.

When older healthy plants are infected, the leaves are not so crinkled as just described. They are much reduced in size and very regular in margin and surface characters, but are not much lighter in color than the healthy leaves.

On the contrary the leaves in the more advanced stages of dwarf have more characteristic symptoms. These symptoms are influenced by climatic factors. The leaf symptoms of succulent growth such as produced under greenhouse conditions or during the vigorous growth of the damp spring months are distinct. Leaves grown under such conditions are larger than those leaves of diseased plants grown later in the summer. They are not deeply crinkled but are wrinkled or bullated in smooth undulations, owing perhaps to the more or less marbled occurrence of the mottling. This marbled mottling is made up of irregular splotching of bronzed-green mesophyll and lighter (chlorotic?) areas. The succulent leaves grown in half shade, as in insect-proof cages, are very finely mottled. Figure 9 shows this mottling, which is the predominating type of chlorosis found in the leaves of dwarfed plants. The contrast with the clear green of the healthy leaf is striking. The leaves shown in figure 9 were grown under cages and were photographed in July. The finely-netted mottling follows rather regularly the netting of the finer veins of the leaf. Tips and margins of the leaflets are usually darker green than their central portions. The margins are not so uniform in outline as those of the healthy leaflets. The mottling of diseased leaves produced in the open during warm dry weather is not very different from that just described, but the leaves are much more rigid—the margins and apices of the leaflets have more of an upright tendency, producing a cupping of the leaflets (Fig. 11). The margins of such leaves are stiff and much less desirable to handle than healthy leaves.

Stem Symptoms.—The stems of dwarfed plants are not mottled or streaked in any way, nor is there any type of necrosis of the bark or inner

tissues. The color of the diseased stems appears as that of normal stems. The spindliness of the canes and shortening of the internodes are the chief characteristics of canes in the early stages of the disease (Fig. 3). Later the canes are extremely foreshortened, characteristically stout and stiff, with extremely short internodes (Figs. 4, 10 and 11). The new canes come later in the spring than those of healthy plants and are fewer in number each year. There are often several buds at each node.

Many canes of dwarf plants have been layered in the fall but they do not readily take root. The spindly canes of the first year may root, but such rooted tips are very weak and seldom grow any sprout.



FIG. 8. The same plant as that illustrated in figure 5, showing the characters of dwarf one year later. Notice the small rounded leaflets and the snubbed tips of the canes. Photographed July 21, 1926.

Fruit Symptoms.—In the less extreme cases of dwarf, fruiting laterals may grow to nearly normal length and set nearly as much fruit as do healthy laterals. The only appreciable abnormality in the flowers is the reduced size of the sepals and petals. The drupelets of the fruit sometimes ripen a little unevenly. The fruit develops to a fair size, but there is a tendency for the drupelets to fall from the receptacle. Some growers say the berries fall apart and become a crumbly mass in the boxes.

Root Symptoms.—The roots of dwarfed plants show no external or internal evidence of the disease except that in its later stages there is a reduction

in the number of the larger roots and finer rootlets. The tips of canes which are layered do not readily take root. The extremely inferior plants so produced do not persist for long.

NATURE OF THE DISEASE

The symptoms and behavior of the dwarf disease of blackberries indicate that it is one of the infectious chlorotic diseases. Sufficient data have been gathered upon which to base a positive statement as to the infectious nature

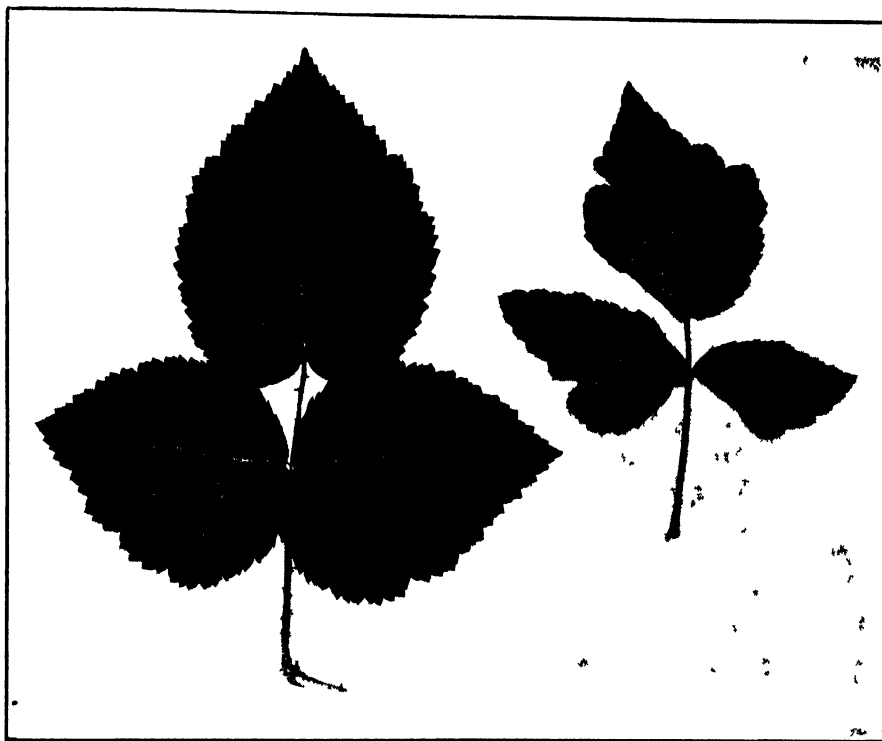


FIG 9. Differences in the size, margins and markings of the leaflets of healthy (left) and dwarfed (right) leaves of the Loganberry. Notice the finely netted mottling of the diseased leaf. Photographed on panchromatic plate, using Wratten A filter.

of the disease. No fungus or bacterial organism has been found associated with the dwarf disease, nor have any foreign cell inclusions been found as yet. Transmission of the dwarf disease, however, has been accomplished by means of aphids (8).

TRANSMISSION OF THE DWARF DISEASE

The general appearance of diseased plants and the gradual spread of the disease year after year led the writer to believe it to be transmitted by some

agent in the field. This supposition led to the first inoculation experiments in 1925. These were repeated in 1926.

Early in April, 1925, twenty-one young tip plants of Loganberry were covered with cages. Vigorous cane growth had already begun. The cages eliminated the possibility of outside infection, particularly from insects, during the spring and summer period of active growth; and the shading aids succulency in the plants, a condition favorable to infection by means of sucking insects.

There is one unfortunate factor which enters inoculation experiments when cages are used to cover perennial plants in the field during only the period of growth. Under such conditions, succulent canes are produced, and it is necessary to lift the cages early enough to allow the plants to harden off before cold weather. This introduces the possibility of infection by outside insects after the cages are lifted. In my experiments there has been no indication of such infection by outside agencies.

The location chosen for the transmission experiments is quite distant from any planting of horticultural varieties of bramble fruits. The cages used in the work are of a convenient size to cover one plant and allow of considerable growth. The cages are 20 inches square and 36 inches high. The lumber for the frames is finished so that it will not catch in the cloth during the process of covering. There is a base board of 1×8 material, and the uprights and upper rim are of 1×3 material. A very good quality of sheeting (LL Caddo) is used to cover the frame. A piece of the cloth for the top, 36×44 inches, has its margins tacked so as to lap about 2 inches over the sides. This allows the top to bag so that a slit about 6 inches long across its center may be tied with a string. This slit serves as a "peek" hole for making observations and doing the necessary work connected with inoculations. A piece of cloth 88×36 inches serves for the sides. The selvage edges are rolled enough to take up the slack and tacked around the top and around the upper part of the base board. The ends are rolled together and tacked along one of the uprights. Then all of the tacked edges are nailed down with lath so that all are insect-proof. The cages are set in place over the plant and the base board is covered with soil up to the lath holding the lower edge of the cloth. Except for size and shape this is the type of insect cage used for caging potato plants by Schultz and Folsom (5) and by McKay of the Oregon Experiment Station.

It is still an open question as to how the dwarf disease is transmitted from plant to plant under natural conditions. The aphid, *Aphis rubiphila* Patch, which is reported by Rankin (4), and *Amphorophora rubi* Kalténback, reported by Wilcox and Smith (7), Berkeley and Jackson (1), and by others, as carriers of the virus diseases of *Rubus* in the Eastern and Middle Western States and Eastern Canada, have not been found west of

the Cascade Mountains in Oregon or Washington. These species of aphids may be in this section but surely they are extremely scarce. Only one collection of each of these species has been reported as taken in Pacific Coast States. Both of these were in San José, California. It is not improbable that the long dry summer seasons, without the intermittent rainfall which is so common in the regions where these species abound farther east, is a factor limiting their propagation here.²

Thus we were at a loss to know what insects to use as a transmitting agency in our experimental work with blackberry dwarf. As aphids, leaf hopper, and such sucking insects are usually the carriers of virus diseases,



FIG. 10. A Loganberry plant in about the third year of dwarf. The longest canes here are about 20 inches long. Notice the rosetted or snubbed ends of the canes. This plant is seven years old.

² During the spring months of 1927, after this paper had been completed, the author found *Amphorophora rubi* on the Himalaya blackberry near Corvallis and Canby, Oregon.

the writer finally decided to make transmission trials with insects usually found on *Rubus* or some other Rosaceous plants. In 1925 aphids were used; in 1926, aphids, leaf hoppers, and tree crickets were given a trial as agencies for dwarf transmission.



FIG. 11. A Loganberry plant in the fourth year of dwarf. The canes were 10 to 12 inches long.

Transmission Trials with Aphis

It was thought most probable that aphids infesting some other Rosaceous host would most readily pass to species of *Rubus*. It was found that the wild sweetbriar rose (*Rosa rubiginosa*) so commonly found in the Willamette Valley was usually infested with aphis. On April 1, 1925, a colony of these was transferred to a cage containing a healthy Loganberry plant. This proved later to be a mixed colony of *Capitophorus tetrarhodus* and *Macrosiphum dirhodum*. This mixed colony was used as the dwarf-transmitting agency in 1925. In about two weeks (April 15) some of the aphids were transferred to a cage containing a Loganberry plant which was extremely dwarfed. This dwarfed plant had been obtained from Gresham, Oregon, early in April.

The 21 healthy Loganberry tips which had been planted out and caged early in April were taken from a disease-free planting which had been under the writer's supervision since the spring of 1924. The original stock was taken early in the spring of 1924 from a Loganberry planting which was

extremely isolated in a valley in the eastern part of Douglas County, Oregon. This whole planting was extremely vigorous and free of any symptoms of virus diseases.

To ten of the cages containing healthy Loganberry plants were transferred aphids from the caged dwarf plant, and to six of the cages aphids from the caged healthy plant were transferred on May 14. From four to ten individuals were transferred in each case. In order to secure the aphids from one plant and place them on another, the leaves of the first plant upon which they were feeding were picked and laid with care on a very young, succulent leaf of the second plant. The next day observations were made and it was found in all cases that the aphids had crawled from the picked, wilted leaf to the leaf of the healthy plant. Observations were made each day for several days in order to detect the first symptoms of disease. On May 20 the first effect of the viruliferous aphids was to be seen. On the leaves of some of the plants there was very slight necrosis along the veins and particularly in the mesophyll between the lateral veins where the aphids were feeding. As the necrotic areas increased, the aphids moved on to more succulent tissues. In a few cases the necrosis was extreme, while in others it involved only the leaf on which the viruliferous aphids were feeding and all of the newer leaves and younger part of the cane above. In one extreme case a young plant was killed to the ground and not until July 17 was there any sign of life. On that day there was observed a tiny new shoot coming from below ground. In late August this had developed into a very dwarfed, chlorotic cane about three inches long. The cage was gradually removed so as to allow the plant time to become hardened to the sunlight. The plant lived until late in December, but died before the spring of 1926, although the winter was extremely mild.

Seven of the ten plants receiving colonies of aphids from the diseased plant showed symptoms of dwarf before the summer months passed. None of the six plants receiving aphids from the healthy Loganberry plant showed symptoms of dwarf and grew normally in the spring of 1926.

After necrosis had been observed there were no abnormalities appearing until subsequent new growth appeared. On June 8 the first dwarf symptoms were noticed in one plant, and soon after that in five other plants. The new leaflets were slightly more rounded than normal, and some crinkling was appearing. By June 20, symptoms of first year dwarf were apparent. On July 17 the plant illustrated in figure 5 was photographed. The symptoms shown there were characteristic of six of the seven plants affected. At this time new canes were starting and the leaves on these were mottled and showed more typical dwarf symptoms. In all cases the canes upon the leaves of which the aphids had been placed did not elongate further. The short laterals they threw out were very ragged appearing, owing to the severe

crinkling and laciniation of the margins of the leaves. The newer canes were elongating considerably by July 28. The leaves were mottled in a splotchy manner by this time, and the whole appearance gave the impression that the entire plant would succumb.

The next year (1926) these plants appeared as shown in figure 8. One year after infection all the six remaining plants showed typical dwarf symptoms.



FIG. 12. Two tips were planted in this hill; one has been infected with dwarf and the other remained healthy.

In the spring of 1926 four caged Loganberry plants were inoculated by transferring to them viruliferous aphids from a dwarfed Loganberry plant. This was done as described above for the 1925 experiments. In this case a pure colony of *Capitophorus tetrarhodus* was used as the transmitting agency. Three of the four inoculations were successful and the progress of the disease in these cases the first year is comparable to that described above. Thus, from the experiments of the two years there have been ten out of fourteen plants to which the disease has been carried by viruliferous aphids, while ten plants to which non-viruliferous aphids from healthy Loganberry plants had been transferred remained healthy.

Transmission Trials with Tree Crickets

The snowy tree cricket (*Oecanthus niveus* De Geer), Race "B," as described by Fulton (3), is so common in plantings of blackberry in Oregon that it was thought to be a possible carrier of the dwarf disease.

Loganberry canes with many egg punctures were collected about May 15, 1926, and some were placed in a cage with a healthy Loganberry plant and others in a cage covering a dwarfed Loganberry plant. The eggs hatched and nymphs matured in these cages. The adult females began ovi-

positing late in July. On August 4, 1926, females which were actively ovipositing were taken after they had deposited several eggs. Two or three of these were transferred to each cage covering healthy Loganberry plants. Four cages received tree crickets which had been ovipositing and feeding on plants affected with the dwarf disease, and four cages received females which had been ovipositing and feeding on healthy Loganberry plants. When these eight plants were examined again in the early part of September, they had all been punctured by the tree crickets, but in no case were there any abnormal symptoms. All the plants appeared healthy late in November. As a result of this experiment the writer does not believe that the dwarf disease is disseminated by the snowy tree cricket.

Transmission Trials with Leaf Hoppers

Leaf hoppers were also tested as possible carriers of the dwarf disease. A large number of a white leaf hopper, probably the rose leaf hopper (*Empoa rosae* L.), were caged on a healthy and on a dwarfed Loganberry plant on July 27, 1926. On August 4 a number of hoppers were transferred to each of eight cages of healthy Loganberry plants, four receiving hoppers from the dwarfed plant and four from the healthy plant. None of these plants had shown dwarf or any abnormal symptoms by November.

Transmission Trials by Other Methods

In 1925 and 1926 a total of eleven healthy Loganberry plants have been inoculated in several different ways with the juice from succulently growing dwarf plants. To obtain this juice the leaves were macerated and ground in a mortar with just enough sterile distilled water to moisten the tissue very slightly. The juice thus obtained was drained off into a bottle and used in the following four ways: (a) Juice was placed on leaves near the growing tips of canes, and punctures were made in the leaf with a needle so that the juice entered the tissues of the healthy leaf. (b) Drops of the juice were placed in the crotch of the leaf petioles next to the axillary bud and the juice was allowed to penetrate the tissues next to the bud through needle punctures. (c) Where canes were forked with two branches, one branch was cut off leaving a stub about 2 inches long. This stub was so placed in a vial containing some of the juice that the cut end could absorb the juice. (d) Juice was injected into the pith and woody cylinder of canes by means of a hypodermic needle.

In no case, as a result of these inoculations, have any abnormal symptoms developed.

In the summer of 1924, buds from dwarfed Logan and Phenomenal plants were budded into healthy Loganberry, Cuthbert red raspberry, and

Munger and Plum Farmer black raspberry. This budding was done by Mr. Lyle Wilcox of the Department of Horticulture, Oregon Agricultural College. None of these buds lived over until the next year owing to winter kill of the canes, but many of them were apparently vigorous until late in the fall. There were no indications, however, of dwarf transmission.

INFLUENCE OF ENVIRONMENT ON DWARF

No particular experiments have been planned to ascertain the relation between climatic or edaphic factors and dwarf symptoms. From the practical standpoint of roguing it should be said that if a plant has ever suffered from dwarf it will retain enough of the symptoms even in winter condition so that the diseased plants may be recognized. The difference between hot weather symptoms and the succulent growth symptoms produced under more favorable moisture and temperature conditions have been discussed in another part of this paper. An erratic elongation of canes of dwarfed plants seldom occurs. Elongation has been noticed during short cool, humid periods, but whether in these cases it was a direct effect of the observed conditions has not been checked experimentally. Increased fertility, such as the addition of horse manure to the soil, has not materially changed the growth of dwarf plants.

COMPARISON WITH SOME OTHER VIRUS DISEASES OF BRAMBLES

As far as the writer is aware, the dwarf disease of blackberries is distinct from the other virus diseases of *Rubus*. It has some symptoms which are similar to some of those of the streak disease described by Wilcox (6) as Eastern blue stem, and the type of mosaic of black raspberry which has been designated as "yellow mosaic" by Dr. C. W. Bennett of the Michigan Agricultural College in an unpublished bulletin. The tips of the canes in dwarfed Logan or Phenomenal plants have the snubbiness which is characteristic of these two diseases in Middle Western States. Black raspberry plants having the red raspberry mosaic may exhibit a similar snubbiness of the tips of the canes, as do also black raspberry plants which become affected slowly with the wilt disease (*Verticillium*). The mottling of the leaves of dwarfed plants is not entirely different from that of yellow mosaic but is entirely different from that of streak.

CONTROL OF DWARF

Since the tips of canes of diseased plants do not readily take root, even when carefully layered, there is little danger of spread of the disease from planting to planting through nursery stock. The only spread in this way might be possible from the transplanting of rooted tips from apparently healthy plants which had been infected very late in the summer or autumn. It is believed, however, that if there were no other means of transmission than the rooting of tips the disease would soon be self-exterminating.

One of the most practical means of prevention of dwarf, as in the case of other virus diseases, is the planting of stock from fields which are free of the disease. Most Loganberry plantings in Oregon and Washington are free of dwarf, but where it exists in low percentages, less than 5 per cent for instance, it is practical and advisable to rogue out the affected plants. From three plantings of Loganberries the disease has been eliminated by two roguing in as many succeeding seasons. These plantings had approximately 1.5, 2.5 and 4.0 per cent infection, respectively.

All plantings of Loganberries where dwarf has been found have been near at least a few plants of the Phenomenals, or the stock from which the plants were taken had been in such a relation to the Phenomenal blackberry. The Phenomenal berry is used so little in a commercial way in Oregon that it should be eliminated from commercial plantings entirely. At least Loganberries should never be grown near Phenomenals.

SUMMARY

1. The dwarf disease of the vining type of blackberry (dewberry), represented especially by the Logan and Phenomenal varieties, was recognized in the Pacific Coast States as early as 1918.

2. The disease occurs wherever the Phenomenal berry is grown and also infects the Loganberry, Cory's Thornless and the Kittitany blackberry. It is most widespread where Loganberries and Phenomenal berries are grown commercially, *i.e.*, from British Columbia south to central California. A map of Oregon is presented to show the known distribution of the disease in this state.

3. The economic importance of the disease is measured by the number of plants affected, for a plant which has had dwarf for one full season is valueless thereafter. Some growers of Phenomenal berries have reported as many as 100 per cent of the plants affected by the third year in plantings which have not been rogued. One planting of Loganberries with 19 per cent of diseased plants in the third year has been found. As a rule, however, the loss through dwarf to the Loganberry industry in Pacific Coast States is very slight, but many individual growers have experienced high enough percentages to make total eradication necessary.

4. Dwarf is characterized in its severe stages by short, stubby canes, the internodes of which are very short. The whole cane has a very leafy appearance. The leaflets are smaller, more rounded, and lighter in color than normal, showing a finely-netted uniform mottling. The fruit develops to a fair size but the drupelets easily fall apart, becoming a crumbly mass in the boxes.

5. Tips of canes do not readily take root when layered and it is believed the disease would soon be self-exterminating if there were no other means of transmission.

6. The dwarf disease is one of the infectious virus diseases as demonstrated by transmission experiments in which a mixed colony of aphids (*Capitophorus tetrarhodus* and *Macrosiphum dirhodum*) found on the wild sweetbriar rose, *Rosa rubiginosa*, were the agents of transmission. In another experiment the disease was transmitted by *Capitophorus tetrarhodus* alone. On the other hand, experiments with the snowy tree cricket (*Oecanthus niveus* De Geer) and leaf hoppers (probably *Empoa rosae*) resulted in no transmission. Juice expressed from macerated diseased leaves was injected into healthy plants in several ways with negative results. When buds from diseased plants were grafted into healthy plants, no disease resulted.

7. Environmental changes have had little influence on symptoms of dwarfed plants.

8. Dwarf of blackberries has some symptoms in common with such other virus diseases of brambles as the streak and yellow mosaic of black raspberries and the expression of red raspberry mosaic when infecting black raspberries. On the other hand, most of the symptoms of dwarf differ from those of other virus diseases of brambles.

9. Preventive measures are (a) to secure stock from plantings which are free of dwarf, (b) to discourage the use of the Phenomenal blackberry as a commercial or home garden variety in districts where the Loganberry is a desirable commercial product, (c) to rogue plantings with small percentages of dwarf, such as five per cent or less.

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STORAGE ROTS OF CRANBERRIES IN THE 1926 CROP¹

NEIL E. STEVENS AND HENRY F. BAIN

INTRODUCTION

The cranberry, *Vaccinium macrocarpon*, offers an unusual, perhaps a unique, opportunity for a nation-wide study of the relative importance of rot fungi under the various climatic conditions found in different parts of the United States. Commercial cranberry growing is concentrated in a few centers. The chief centers are, however, widely separated. Certain superior native varieties selected in the past have been used almost exclusively in planting the later bogs, with the result that the same varieties are found to some extent in all cranberry sections. Except in New Jersey, field rots are of relatively small importance; whereas storage rots, caused by fungi which infect the berries in the field, are of paramount importance. Cranberry fungi have been studied intensively by Dr. C. L. Shear and his associates for 25 years. This does not mean, of course, that all cranberry fungi are known. Several unpublished rot fungi have already been found and their descriptions are in manuscript form. Many more will probably be discovered.

The writers have attempted this year to measure quantitatively the actual storage rots caused by the various fungi in cranberries from different growing regions, and to obtain a better idea of the succession of these rots as they develop under ordinary storage conditions. It is hoped to continue the experiment for several years in order to accumulate a fund of information which should prove valuable in future studies along these lines. Because of the amount of work involved, only two varieties could be used in the tests. No given lot can be considered as truly representing the crop in an entire growing section, but in spite of its limitations, in its present stage this is the most complete study of cranberry storage rots ever made, and as such seems to warrant brief presentation.

As in all our work on cranberry diseases, we are indebted to several cranberry growers and others for assistance of various kinds. In this case we are particularly under obligation to the American Cranberry Exchange for furnishing storage facilities in Chicago, and to Dr. H. J. Franklin, who supplied the Massachusetts berries for the experiment.

¹ Investigation conducted cooperatively between the Office of Fruit Diseases, Bureau of Plant Industry, and the Wisconsin Department of Agriculture.

METHODS

Berries were used from four states: Massachusetts, New Jersey, Wisconsin, and Oregon. The last was considered as representing the cranberry region of Washington and Oregon, most of which is located near the mouth of the Columbia River in those two states. One other locality was considered, *viz.*, Long Island, N. Y., but was rejected, as it lies between Massachusetts and New Jersey, which are themselves nearer together than either is to Wisconsin.

The varieties chosen for the tests were the Howes and the McFarlin. The Howes, the standard late variety of Massachusetts and New Jersey, is now being extensively planted in Wisconsin. Although planted in Washington and Oregon early in the development of the industry there, it has not proved particularly satisfactory, and the acreage has been reduced to some extent. The McFarlin is the most important of the so-called fancy varieties. It is the most extensively grown variety in Washington and Oregon, and ranks second only to unselected native vines in Wisconsin. In Massachusetts it is the third variety in importance, but it is grown in only a few places in New Jersey.

The berries were all shipped to Chicago by freight or express without refrigeration. They were shipped in ventilated half-barrel boxes without cleaning or sorting, and were stored together in the shipping boxes until needed for the experiment. About the middle of each month, October to January inclusive, one box of each of the eight lots was opened and a peck of sound berries was sorted out by hand. These eight pecks of sound berries were then stored together in the commercial warehouse of the American Cranberry Exchange in Chicago, under the ordinary storage conditions prevailing in the warehouse, for a period of two weeks. At the end of this period the peck samples were again carefully sorted, and the percentage of berries which had spoiled in the two-weeks period was determined by a numerical count of approximately one-fourth of all the berries in each lot. The spoiled berries were shipped at once to Washington, where cultures were made from 100 rotted berries from each lot or from all the berries if there were less than 100. With the exception of the last two tests, most of the spoiled berries in each sample were cultured; in the last tests, however, too many berries spoiled to permit this. Cultures were made by sterilizing the surface of a berry with mercuric chloride solution and transplanting a portion of the pulp to a culture medium. The tubes were kept in the greenhouse until the fungi fruited or developed sufficiently for identification.

The material for the last three lots of Wisconsin Howes was accidentally lost after it had become impossible to duplicate the samples; consequently the data for these lots are lacking in table 1.

TABLE 1.—Percentage of loss due to different fungi and other causes in the Howes and McFarlin varieties of cranberries from Massachusetts, New Jersey, Wisconsin and Oregon, in the 1926-27 Chicago storage test*

Test no.†	Sterile breakdown		<i>Fusicoccum putrefaciens</i> Shear		<i>Acanthorhynchus vaccinii</i> Shear		<i>Guignardia vaccinii</i> Shear		<i>Gloeosporium</i> sp.		<i>Ceuthospora lanata</i> Shear		<i>Phomopsis</i> sp.		Total per cent spoiled	
	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin	Howes	McFarlin
Massachusetts																
1	0.27	0.24	0.09	0.22									0.42	0.14	1.3	1.0
2	0.48	0.42	0.75	0.36			0.03		0.19	0.11				0.08	1.6	1.0
3	0.53	0.92	0.44	1.70					0.11	0.09					1.1	4.6
4	2.82	2.50	3.23	1.66		0.13	0.24						0.53	0.49	6.7	4.9
5	3.90	3.57	3.98	4.04			0.16								8.3	8.1
New Jersey																
1	0.54	0.60	0.49	0.20	0.49	2.60	0.27	1.00	0.05				0.15	0.60	2.7	10.0
2	0.53	0.43	1.53	0.06	0.19	0.61	1.98	1.22		0.12					4.4	2.9
3	0.77	1.73	2.04	2.90	0.06	0.20	0.12		0.12	0.10			0.13	0.59	3.2	5.1
4	2.59	3.36	4.00	5.64			0.07	0.39						0.28	7.4	9.9
5	3.02	5.10	5.30	8.26		0.14									8.4	13.8
Wisconsin																
1	0.24	0.78	2.64	3.11	0.09	0.05						0.19		0.19		6.4
2	0.68		3.16	3.69			0.07								4.7	5.2
3		0.58		5.00		0.25	0.32							0.07	4.2	3.8
4		3.35		4.90		0.09	0.34									8.6
5		6.72		9.73												16.8
Oregon																
1	1.30	1.26	0.80	0.48		0.09					1.40	0.48	0.60	0.33	5.0	3.0
2	0.90	0.09	1.32	0.73							0.30	0.18	0.08	0.05	2.8	1.0
3	2.13	1.23	0.99	0.01	0.04		0.04						0.53		3.8	1.3
4	2.94	5.50	0.28	1.82		0.08		0.23						0.43	7.9	3.5
5	13.75	10.55	1.37	2.00		0.43	0.15	0.86			0.15				15.4	14.3

* The five lots under each heading represent the amount of rot developing in samples of sound cranberries which were sorted by hand at the beginning date of each test period and stored as follows: 1.—Oct. 15, 1926. Not stored. Condition of berries upon arrival in Chicago. 2.—Stored from Oct. 15 to Nov. 3, 1926. 3.—Stored from Nov. 15 to Dec. 1, 1926. 4.—Stored from Dec. 15, 1926, to Jan. 3, 1927. 5.—Stored from Jan. 17 to Feb. 4, 1927.

RESULTS

The results are given in table 1, which of course does not include numerous fungi which occurred only a few times. The figures presented were calculated in the following manner: first, the percentages of fungi and sterile tubes from a given lot of berries were determined in relation to the total number of spoiled berries of that lot from which cultures were made. The resulting set of figures of course also showed the corresponding ratios of spoiled berries which had been rotted by the fungi indicated. The figures thus obtained were then multiplied by the percentage of total spoilage or decay that had developed in the stored lot of berries, the latter value having been determined when the sample was sorted. This product, given in table 1, thus represents, within the limits of error under the conditions of the test, the actual amount of destruction in each lot of berries caused by each fungus. The results are shown graphically in figures 1 and 2.

The method of calculation used above is open to some theoretical objections, but it does bring the results from the various localities together on a comparable basis, and shows with some degree of accuracy the approximate amount of loss caused by the various fungi in the test samples. The small size of many of the figures will be readily understood when it is remembered that the total spoilage in the brief period of two weeks is usually not large, and that this spoilage is due to several different organisms as well as to other causes.

The first series of results, number 1 under each lot in table 1, represents the condition of the berries on October 15, a few days after they reached Chicago. The rotten berries present on this date of course include any which may have been rotten when harvested together with those which developed rot in transit. Since the environment of the different lots was not uniform during this time, the results in the first series are not entirely comparable to those of the other four series.

The most striking fact brought out in the table is the preponderant importance of end rot, caused by *Fusicoccum putrefaciens* Shear, in berries of both varieties from all four regions. End rot is by far the most important of storage rots of cranberries from any region, at least under the conditions of our 1926 storage tests. Its importance increased markedly in the later tests, while most of the other fungi tended to decline with the advance of the season. It should be pointed out, however, that the storage temperatures later in the season were much more favorable to the development of the end rot organism than to that of most of the other fungi.

Guignardia vaccinii Shear and *Phomopsis* sp. appeared to some extent in berries from all four regions, although *Guignardia* is of course much more abundant in New Jersey berries than in those from any other state. *Acanthorhynchus vaccinii* Shear, the second or third most important rot fungus

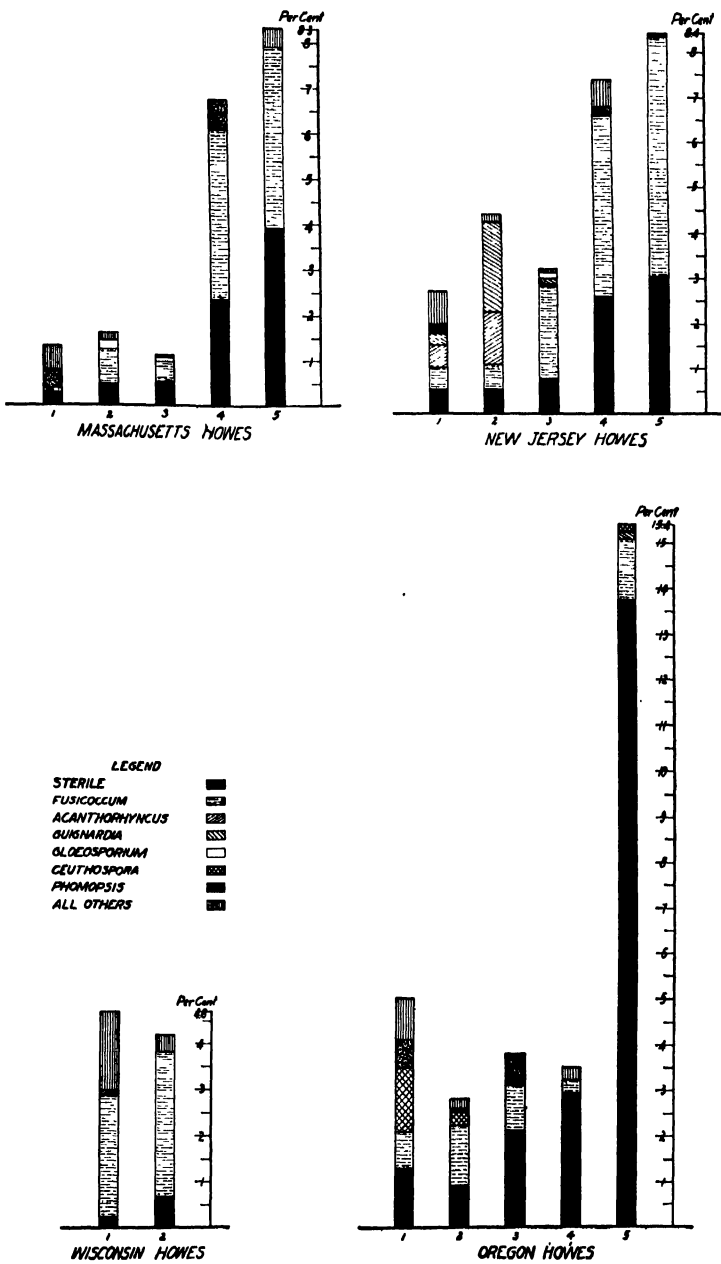


FIG. 1. Percentage of loss due to different causes in the 1926-27 Chicago cranberry storage tests. Columns 1, 2, 3, 4, and 5 in each graph represent the spoilage occurring prior to October 15, between Oct. 15 and Nov. 3, between Nov. 15 and Dec. 3, between Dec. 15 and Jan. 3, and between Jan. 17 and Feb. 4, respectively.

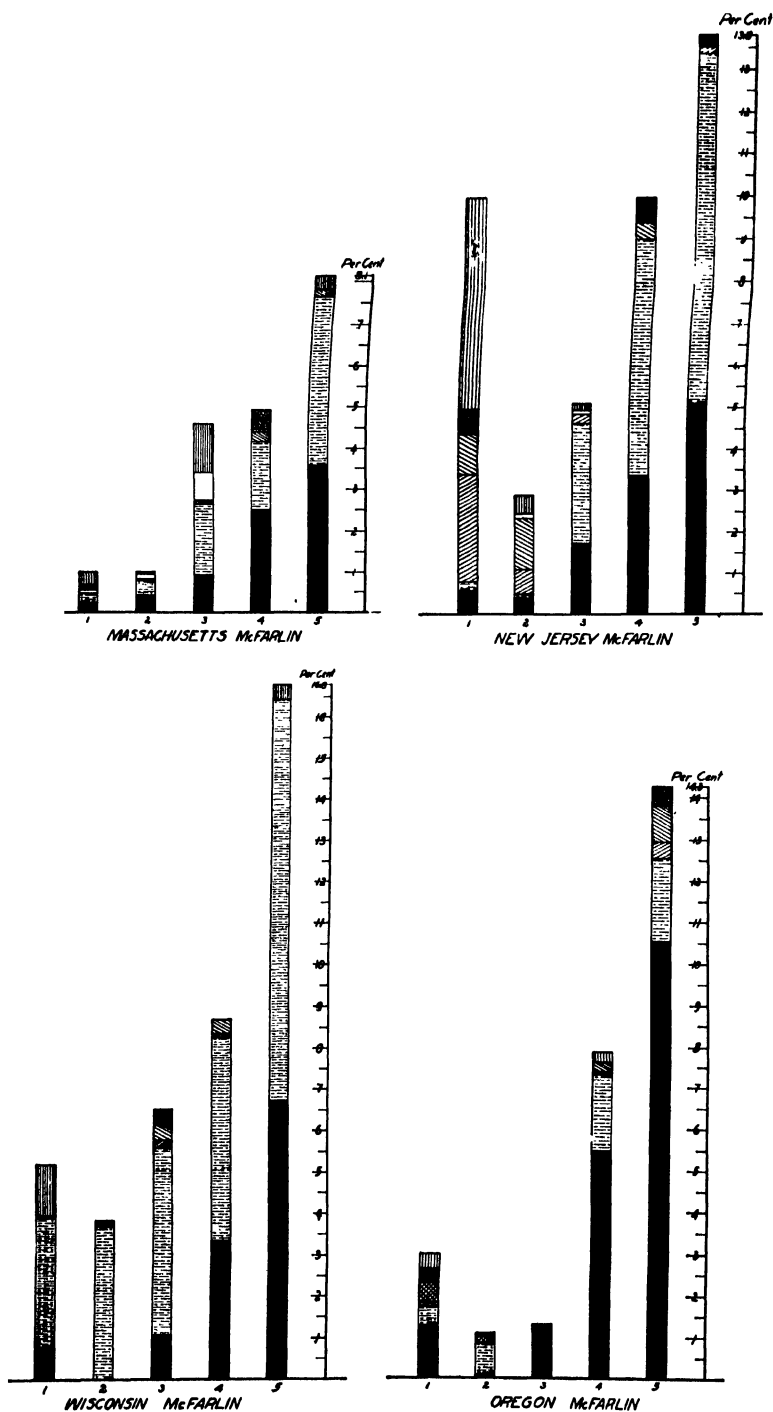


Fig. 2. Percentage of loss due to different causes in the 1926-27 Chicago cranberry storage tests. See figure 1 for legend.

in New Jersey, failed to appear at all in Massachusetts berries. *Gloeosporium* sp. was found only in berries from Massachusetts and New Jersey; and *Ceuthospora lunata* Shear, which is of considerable importance in Oregon, was not found at all in Massachusetts or New Jersey, and only once or twice in Wisconsin material.

Perhaps the most interesting feature of all is found in the first column—the spoiled berries without evidence of fungus infection. This type of breakdown showed the same tendency as end rot to increase with the advance of the season, and was usually present in an amount equal to or greater than the latter. The writers are inclined to consider the relative abundance of berries which spoiled without evidence of fungi as an indication of the inherent strength of the berries. It seems probable that these sterile berries either died what might be termed a natural death, that is of old age, or that they were smothered. In either case the relative number of spoiled berries which proved sterile would indicate the relative strength of the berries of that lot. In this connection it should be noted that, while all the berries were held under identical conditions beginning only a few weeks after harvest, there was a distinct variation, characteristic to both varieties, in the amount of the breakdown which appeared in berries from the different localities. If we are correct in our interpretation of this type of spoilage, there is a decided difference in the inherent strength of the same varieties of cranberries when they are grown under diverse climatic conditions.

SUMMARY

End rot, caused by *Fusicoccum putrefaciens* Shear, was found to be decidedly the most important storage rot fungus in samples of both the Howes and the McFarlin varieties of cranberries grown in the four principal cranberry regions of the United States in 1926.

Several other cranberry rot fungi, together causing a smaller amount of rot than *Fusicoccum*, varied in abundance in the different growing regions.

End rot developed in increasing quantity as the storage season advanced. All other storage rot fungi either decreased in importance with the advance of the season or occurred in about the same degree throughout the season.

A progressively increasing percentage of berries in all samples tested spoiled without evidence of fungous infection. The amount of this type of spoilage varied with the locality in which the berries were grown. Spoilage of this type is believed to be an indication of the inherent strength of the berries as grown under the prevailing climatic conditions.

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE,

AND

WISCONSIN DEPARTMENT OF AGRICULTURE.

SAND BURN OF PECAN SEEDLINGS

J. B. DEMAREE

Sand burn, a disease of pecan seedlings, was observed by the writer for the first time in a nursery near Cairo, Georgia, during the summer of 1922. Undoubtedly the disease had been present in southern pecan nurseries for several previous years. Mr. O. M. Hadley, Junior Meteorologist, United States Weather Bureau, Thomasville, Ga., informed the writer that he had observed this type of injury in a small nursery in 1912. Since 1922 the writer has seen the disease in nurseries in, or has received specimens from, the following localities: Milledgeville, Shellman, Phlema, Albany, Baconton, Thomasville, and Cairo, Georgia, and Monticello, Florida. The disease was exceptionally prevalent during June and July, 1924.

DESCRIPTION

Sand burn is found in pecan nurseries principally during their first year of growth and for the most part within the period May to July inclusive. The first evidence of the trouble on seedlings that are one to three months old is a small brown spot on the side of the stem which is exposed to the west or the southwest at a point either at or slightly above the soil surface. Following the initial symptom, *i.e.*, the appearance of a brown spot on the side of the stem exposed to the sun's rays, the cortical tissues lying just under the spot collapse and a sunken area results. At the same time the color of the injured surface changes to dark brown or black. Coincident with the breaking down of the cortical tissues lying under the spot, the lesions become somewhat elongated, averaging about one-half inch in length, and frequently extend around the stem, forming a distinct constriction which cuts off the upward flow of sap. The portion of the stem above the lesion dies as a result of complete girdling (Plate XXIV, A). The girdling process appears to extend over a period of several days; consequently the aerial portion of the seedling dies gradually. Injured plants may be detected by certain symptoms several days before they die. The leaves of a severely injured seedling first take on a reddish cast, and about the same time the edges of the leaves roll upward toward the mid-vein. Later the leaves may assume various positions in reference to the axis of the stem (Plate XXIV, B). Some point downward, others stand out at right angles to the stem, and still others retain their normal position, pointing upward and outward. When the lesion completely girdles the seedling, the leaves die

and turn black but do not separate from the stalk. Fragments of the leaves, especially the petioles and rachises, often adhere to the dead stems several months after death. Sometimes the lesions do not extend entirely around the stem, there being a narrow strip of uninjured cortical tissue. An injury of this type usually results in the formation of a callus or swelling immediately above the lesion. Seedlings of this kind may recover entirely and make fairly healthy trees, but more frequently, especially those that have been almost completely girdled, break off during the course of the season. The portion of the seedling below the girdled area does not die as a direct cause of the original injury but sends out sprouts at points on the stalk from one-half to one and one-half inches below the lesion. The fate of such secondary sprouts seems to be determined largely by weather conditions at the time of their emergence from the soil or shortly afterwards. Provided the soil is moist and the air temperature is not excessively high, they usually grow and live through the stage during which they would be susceptible to heat injury. However, these secondary sprouts frequently die when their emergence from the soil is followed by an excessively hot period.

A second form or manifestation of sand burn is that the terminal buds of young plants are killed as they attempt to push through the hot surface layer of soil. This type is more likely to occur in nurseries where the nuts were planted late and the young seedlings emerge in May or June. As many of the injured plants fail to appear, the thinness of the stand, where the injury is severe, is often attributed by growers to failure of the nuts to germinate. As in the case of the girdle type of injury, the portion of the seedling below the point of injury will send up, in some cases, five to seven shoots, simultaneously or successively, which die as they reach the hot surface. Occasionally, one of these shoots manages to grow above the surface, uninjured, but it makes a weak plant of little or no value for propagating purposes (Plate XXIV, C).

FACTORS WHICH SEEM TO BE RESPONSIBLE FOR SAND BURN

Although no experiment has been conducted in an endeavor to produce the symptoms of sand burn artificially, field evidence and laboratory examinations of the lesions indicate that the causative factor is of non-parasitic nature. In general appearance the lesions resemble damping-off diseases attributed to species of *Pythium*, *Corticium*, or *Fusarium*. However, cultures from 169 affected seedlings made during July and August, 1924, failed to develop any damping-off fungi, or any other recognized plant parasite. Most of the cultures developed *Mucor*. Possibly parasitic fungi take some part in the girdling process after the plant tissues have been injured. The fact that the injury usually first manifests itself on the south

or west side of seedlings and during a period of high atmospheric temperatures suggests that high temperature is the predisposing factor. In support of the high-temperature theory, it should be recorded that the most extreme cases of sand burn in pecan nurseries have occurred either on westerly slopes or on dark-colored soil. The highest mortality of pecan seedlings observed by the writer occurred during June, 1924, when the mean maximum shade temperature for the month was 90.7° F. There were 18 days during this month when the temperature was 90° F. and above. During a continuous period of 13 days in this month the daily maximum shade temperatures ranged from 90 to 98° F.¹

The temperature of the surface layer of dry, sandy soil of the Coastal Plain Region becomes extremely high on hot clear days. One test recorded the temperature of the air one inch above the soil at 119° F., and one inch below the surface at 113° F., but when the thermometer was placed directly on the surface it registered 126° F.

There seems to be an intimate relationship between grade or quality of nuts planted and severity of sand burn. The writer is indebted to Mr. J. L. Pelham, in charge of the United States Pecan Experiment Station, Phlema, Ga., for valuable information on this question. The Phlema nursery was planted in February, 1924, with seed which had been graded into three classes according to size. In May following the planting of the nuts the weather was exceedingly dry and hot, and heavy mortality of the seedlings resulted from sand burn.

The writer visited this nursery on June 6 and again July 17, 1924. While many seedlings were dead and others dying in all plots, there was a marked difference between the stand in the plots planted with high grade nuts and those planted with low grade. Invariably, nuts graded as No. 1 produced more vigorous plants with a smaller percentage of sand burn. A poor stand resulted from planting the lower grade of nuts, owing not so much to low percentage of germination as to death of the terminal buds, either as the plants pushed through the soil surface or became girdled after they had attained a height of from four to six inches.

Sand burn seems also to be associated with late planting. Ordinarily pecan nuts are planted in November and December. Sometimes on account of labor troubles, unfavorable weather conditions, or difficulty of procuring seed, nurserymen delay planting until February or even March. Early planted nuts germinate during March and April, and grow vigorously during the spring months, so that by the time the hot dry weather of summer approaches the young seedlings seem to have grown beyond the stage of susceptibility to heat injury. On the other hand, if the nuts are planted

¹ Climatological Data, United States Weather Bureau Station, Thomasville, Ga.

during late winter or early spring, the seedlings may not appear above the surface of the soil before April or May and consequently are exceedingly tender and susceptible to sand burn during the normally hot dry weather of May and the first half of June.

Sand burn is frequently the most important contributing factor to poor stands of seedlings in pecan nurseries. A deficiency of soil moisture, especially in soils where the sand is from two to several feet deep, may delay the germination of early planted nuts until late spring or early summer. Should the soil surface be excessively hot during the period in which the tips of the seedlings are approaching the soil surface, many would be killed before or during emergence.

ECONOMIC IMPORTANCE

The loss of young pecan seedlings caused by sand burn varies from year to year, and also varies greatly during the same year in different localities. The loss is frequently of very little importance in nurseries planted on level soil of light or moderate fertility and water-holding capacity. During seasons in which high atmospheric temperatures occur during May and June the loss may amount to 50 or 75 per cent on deep, sandy soil, or on hillsides with a western exposure.

HEAT INJURY TO OTHER PLANTS

Hartley² reported serious injury to coniferous seedlings which he attributed to excessive temperatures. He recorded a temperature of 126° F. in the surface layer of soil in coniferous seed beds even under half shade of lath frames. Münch³ described an injury to tree seedlings similar to sand burn of pecan seedlings, and attributed it definitely to excessively high temperature at the soil surface. Other investigators, both in this country and in Europe, have reported stem girdle and other forms of injury to seedlings of trees and herbaceous plants.

PREVENTIVE MEASURES

The planting of high grade seed during early winter, the avoidance of deep sandy soils which are subject to overheating, and the selection of a nursery site not having a westerly exposure greatly lessen the danger of heavy loss by sand burn in pecan nurseries.

² Hartley, Carl. Stem lesions caused by excessive heat. *Jour. Agr. Res.* 14: 595-604. 1917.

³ Münch, E. Hitzeschäden an Waldpflanzen. *Naturw. Ztschr. Forst. Landw.* 11: 557-562. 1913.

SUMMARY

1. Sand burn of pecan seedlings, which has occurred in southern pecan nurseries for several years at least, is herein reported for the first time.

2. Seedlings in pecan nurseries develop the trouble during the first year of their growth. There are two well-defined types of sand burn. The most striking type is a girdling of the stems at a point near the surface of the soil, which results in the death of the seedlings. The leaves of the dead seedlings turn black but do not fall off at once. In the second type of injury the buds of the young seedlings die as they reach the hot surface layer of the soil. This type of injury, though causing greater mortality of seedlings, is less conspicuous than the first type described.

3. Excessively high temperature at the soil surface is believed to be the direct cause of sand burn of seedlings, but inferior seed and late planting are contributing factors.

UNITED STATES DEPARTMENT OF AGRICULTURE,
THOMASVILLE, GEORGIA.

DESCRIPTION OF PLATE XXIV

A.—A two-months-old pecan seedling completely girdled near the soil surface by sand burn injury. Constriction at point of arrow.

B.—Counting from the left, the first, second, fifth, and sixth seedlings have been killed by high soil temperature. The third was dying. Note the distortion of the leaves and their position in relation to the axis of the stem. The fourth plant was uninjured.

C.—Pecan seedlings injured by hot surface soil. The original growing point was killed, whereupon several secondary sprouts developed and in most cases were also killed at the apex by excessive heat.



THE RELATION OF INSECTS AND WEATHER TO THE DEVELOPMENT OF HEART ROT OF CELERY¹

J. G. LEACH

A heart rot of celery, apparently the same as that described by Wormald (4, 5) and caused by *Bacillus carotovorus*, has been prevalent and destructive in the celery bogs near St. Paul, Minnesota. The disease is characterized by a brown, mushy decay of the small heart leaves. When conditions are favorable, the growing point is killed within two or three days. The stem then begins to lengthen rapidly, and the plant becomes commercially worthless (Fig. 1). Frequently as many as 50 per cent of the plants in a field may be so affected, and on one occasion the writer saw a field that was a total loss due to the disease.

The experience of the growers, and the observations of the writer over a period of four years, show that weather conditions have a profound influence on the development of heart rot. This influence, however, is not what one would expect of a disease caused by *Bacillus carotovorus*. This organism is known to be especially susceptible to desiccation and, as a rule, is most destructive under conditions of high humidity. Destructive outbreaks of heart rot, however, occur only in hot dry weather and are usually most destructive on the drier bogs. Even after the disease has become prevalent throughout a field, a period of rainy weather will apparently check its development completely.

In 1923, investigations were started with the view of finding an explanation for this apparent anomaly. Frequent and minute observations of the development of the disease were made throughout the growing season. The first indications of the disease are the appearance of small, brown spots near the tips and margins of the partly unfolded heart leaves. A careful examination of such leaves with a hand lens has always revealed one or more small dipterous larvae actively working in or about the spots. As the disease progresses, the larvae increase in size, but, on account of the mass of decayed material present, they are evident only on close examination. A large number of the larvae were reared to maturity, and representative specimens of the resulting adults were kindly identified by Dr. J. M. Aldrich of the National Museum. Several species were obtained, but the most prevalent were *Scaptomyza graminum* Fall. and *Elachiptera costata*

¹ Published with the approval of the Director as Paper No. 714 of the Journal Series of the Minnesota Agricultural Experiment Station.

Leow. Both of these species have been reported as leaf miners of cultivated plants. The following account of *Scaptomyza graminum* is taken from Frost (3, p. 93).

"*Scaptomyza graminum* is a European leaf-mining species which has been mentioned many times in American literature. Howard (1896) reports it as occurring in decayed and fermenting fruit. Other writers speak of it as a leaf-miner. Sturtevant (1916) established beyond a doubt the dual habit of the species. Its operations are marked by a large, shapeless blotch, with smaller winding galleries conducting to it.



FIG. 1. Two celery plants affected with heart rot. The outer leaves have been removed, exposing the central stalk. This stalk has greatly elongated owing to the destruction of the growing point by the soft rot. Such plants are commercially worthless.

The species has a long list of host plants. . . .

Scaptomyza graminum has been collected in England, France, Germany, and Denmark, as well as in North America. In America it ranges from Canada southward to Georgia, and from the Atlantic coast westward to Minnesota."

Elachiptera costata also has been reported as attacking radish and melon roots (1).

In watching the early stages of development of the disease, small white eggs (Fig. 2) were observed on neighboring healthy leaves and on the heart leaves of yet healthy plants. Some of these healthy leaves which were bearing eggs were cut and placed on moist filter paper in a petri dish. Similar leaves bearing no eggs were cut and placed under similar conditions. The eggs hatched during the following 18 hours, and when the leaves were next examined the maggots were found about one inch from the egg shells, burrowing into the leaves. A small brown discoloration was already evident. This rapidly enlarged, and after five days the leaves were almost completely decayed by typical soft rot. The control leaves remained fresh and turgid (Fig. 2).

Typical cultures of *Bacillus carotovorus* were isolated from the decayed leaves. The larvae were reared to maturity and identified as *Scaptomyza graminum*. The experiment was repeated later, using eggs of *Elachiptera costata* with the same results.

Shortly after an outbreak of the disease in the field in 1923, there occurred a period of prolonged rainfall. Heart rot disappeared from the field and the growers suffered no more losses. Soft rot, however, was found to be just as prevalent as ever, but during rainy weather it was confined to the older outer leaves and petioles. Since these are normally discarded by the grower at harvest, no appreciable loss is felt.

These observations show, then, that the influence of the weather is not so much on the prevalence of soft rot as on the portion of the plant that is attacked. When the heart leaves are attacked and the terminal bud is destroyed, the plant is usually a total loss, while many of the old outer leaves may be destroyed without appreciable loss. A study of the habits of the insect shows why the disease affects the heart leaves in hot, dry weather only. Eggs of the insect are normally deposited in places where the relative humidity is high. In hot, dry weather nearly all of the eggs are found on the younger leaves near the heart of the celery plant. None are to be found on the older, outer leaves. In rainy weather they are found on the outer leaves as frequently as on the inner leaves. Furthermore, when the larvae emerge from the egg, they immediately search for a moist place. In the hottest and driest weather the small heart leaves are always

moist, while the outer leaves are dry. The young larvae, therefore, in dry weather soon find their way to the heart leaves, while in rainy weather they may develop equally as well on the outer leaves. It is also possible that in rainy weather the heart leaves are somewhat more resistant to the decay,

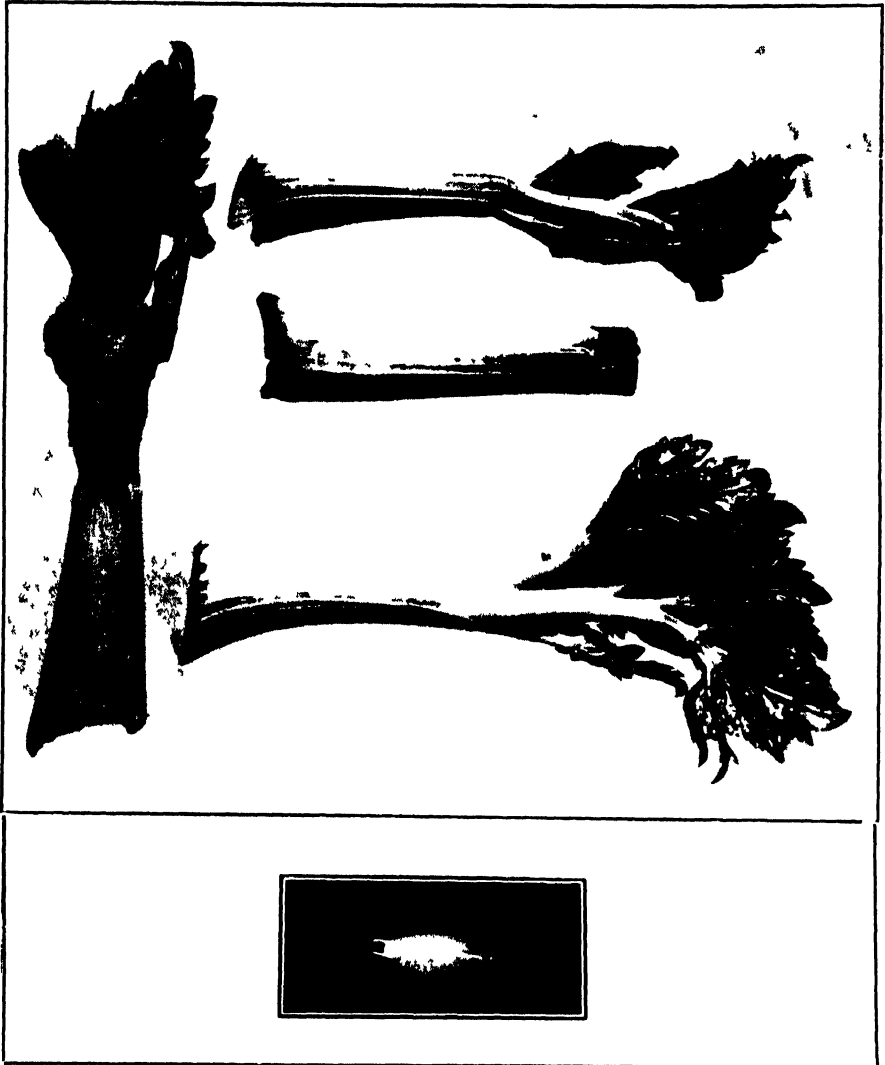


FIG. 2. Young leaves and stems removed from a healthy celery plant and kept in a moist chamber for three days. Those on the right bore one or more eggs of *Scaptomyza graminum*, while the leaf on the left was free of eggs. The decay of the leaves on the left developed as a result of inoculation with bacteria by the larvae which hatched from the eggs and burrowed into the leaves. The insert is a photograph of an egg of *Scaptomyza graminum*, approximately 28 \times .

because heart leaves showing incipient infection have been observed to out-grow the decay.

It was thought at one time that heart rot might be a secondary condition following the non-parasitic trouble known as black heart. This, however, is not the case. Foster and Weber (2), in describing black heart as it occurs in Florida, state: "Blackheart, as found in the South, is distinctly different from 'heart rot' or 'crown rot' which have been reported from northern states and Europe. The latter is a typical soft rot, evidently caused by bacteria of the *Bacillus carotovorus* group, and the former or Florida blackheart is not a soft rot but a typical dry rot followed by premature death of the growing crown." Nothing comparable to black heart has ever been observed by the writer in Minnesota.

SUMMARY

The experiments cited above show that *Scaptomyza graminum* and *Elachiptera costata* are common agents of inoculation of celery heart rot. It is possible that other similar insects may also be equally effective, but it has been definitely proved for these two species only. In addition, these insects in all probability are the chief agents of dissemination, as the adult flies commonly feed on the decaying tissues and have every opportunity to carry viable bacteria from diseased plants to the healthy leaves on which they deposit their eggs.

During the summer of 1926 attempts were made to control the disease by dusting the heart leaves with an insecticide, but shortly after the plants were dusted a prolonged period of rainy weather occurred and the disease did not develop even on the undusted rows. Therefore no results were obtained.

UNIVERSITY FARM,
ST. PAUL, MINN.

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NOTES ON THE *CERCOSPORELLA* LEAFSPOT OF CHINESE CABBAGE IN MASSACHUSETTS

W. H. DAVIS

The *Cercospora* leafspot was first observed on the leaves of Chinese cabbage during September, 1925, and was collected from outdoor plats during each week until January, when the plants were covered with snow. The disease reappeared in the fall of 1926 but was less prevalent and injurious to the crop.

The injury was most severe during the cool weather in autumn: some plants were entirely killed, although others were only slightly affected. The injury was not severe during the main growing and harvesting season.

The initial symptoms appeared in two distinct forms: (a) as minute white, or dilute brown, circular, papery lesions both on young and old leaves—these lesions were definitely delimited, and often dead, dark brown veinlets crossed their surfaces; (b) as minute, yellow, circular spots which spread until the centers were papery, white areas crossed by the dark brown leaf veinlets (Plate XXV, B, 3-4). Fully developed lesions on living leaves were circular and somewhat delimited by the large veins which branched from the midrib (B, 1-2). These lesions averaged 8 mm. in diameter but sometimes coalesced, in which case they appeared as large, dried, irregular, dead areas (A, 5). The margins of the lesions were generally bordered by a dark green area which was seldom raised but appeared more conspicuous in dried herbarium material. White, circular, papery lesions crossed by a network of dark brown veinlets were unmistakable symptoms of this disease.

In the lesions there were two kinds of mycelium: (a) small hyphae, 2-4 microns in diameter, which were mostly intercellular and located near the margins, and (b) hyphae with large cells, $5-6 \times 7-34$ microns, generally vacuolate and bearing large nuclei. These hyphae were located in the dead, papery, white portion of the lesion.

Sclerotia-like bodies consisting of entwining masses of mycelium formed beneath the epidermis of the leaf. Very short and sometimes indistinguishable conidiophores originated from these mycelial masses. Structures which appeared like conidiophores were unbranched, mostly cespitose but sometimes single; mostly erect but sometimes detached and prostrate on the surface of the leaf; hyaline, averaged 3 microns in diameter and varied from 0 (indeterminate) to 13 microns in length. It was not decided whether these were conidiophores, immature conidia, or cells of broken conidia which remained attached to the sclerotia-like bodies. However, some conidia origi-

nated from hyphal strands which had emerged from the leaf tissues by penetrating the epidermis. The conidia were hyaline, 1- to 7-, mostly 3- to 4-celled, elongate-cylindrical, seldom fusiform and constricted at the cross-walls, mostly curved but often straight. Limits for the measurements of 100 conidia: $1.5-3.5 \times 22-100$ microns; standard, 2.5×56 microns.

Beets, celery, parsnips, carrots, and common cabbage were inoculated with conidia from Chinese cabbage to determine the hosts of this fungus (series I). Beets, celery, and parsnips growing in nearby plats were infected with *Cercospora* species, so reciprocal inoculations were made with conidia from Chinese cabbage and each of the other three hosts (series II).

In series I, five different inoculations were made on each of the plants which were cultured under controlled greenhouse conditions. The Chinese cabbage plants were the only ones in this series that became infected. In series II, three different multiple inoculations were performed. In all inoculations the Chinese cabbage remained healthy unless inoculated with conidia from Chinese cabbage. The *Cercospora* from Chinese cabbage did not infect the other hosts inoculated.

In view of the fact that species of *Cercospora* which infect Chinese cabbage and various other crucifers have not been definitely described and comparatively few inoculations reported at the present time, it is difficult to determine species and races definitely. The negative results from some of the inoculations previously described might have been due to physiological specialization (races).

From the descriptions at hand and the examination of herbal material available, it appears that *Cercospora bloxami* B. and Br., *Cercospora albo-maculans* E. and Ev., and *Cercospora brassicae* Jaap. are synonymous with *Cercospora albo-maculans* E. and Ev. (Saccardo, "Sylloge Fungorum" 11: 606. 1895).

The genus and species of the organism which causes this disease on Chinese cabbage should be reported as *Cercospora albo-maculans* (E. and Ev.) Sacc. until further cultural studies and comparisons of organisms from a larger number of hosts present data to the contrary.

DEPARTMENT OF BOTANY,

MASSACHUSETTS AGRICULTURAL COLLEGE,

AMHERST, MASSACHUSETTS.

DESCRIPTION OF PLATE XXV

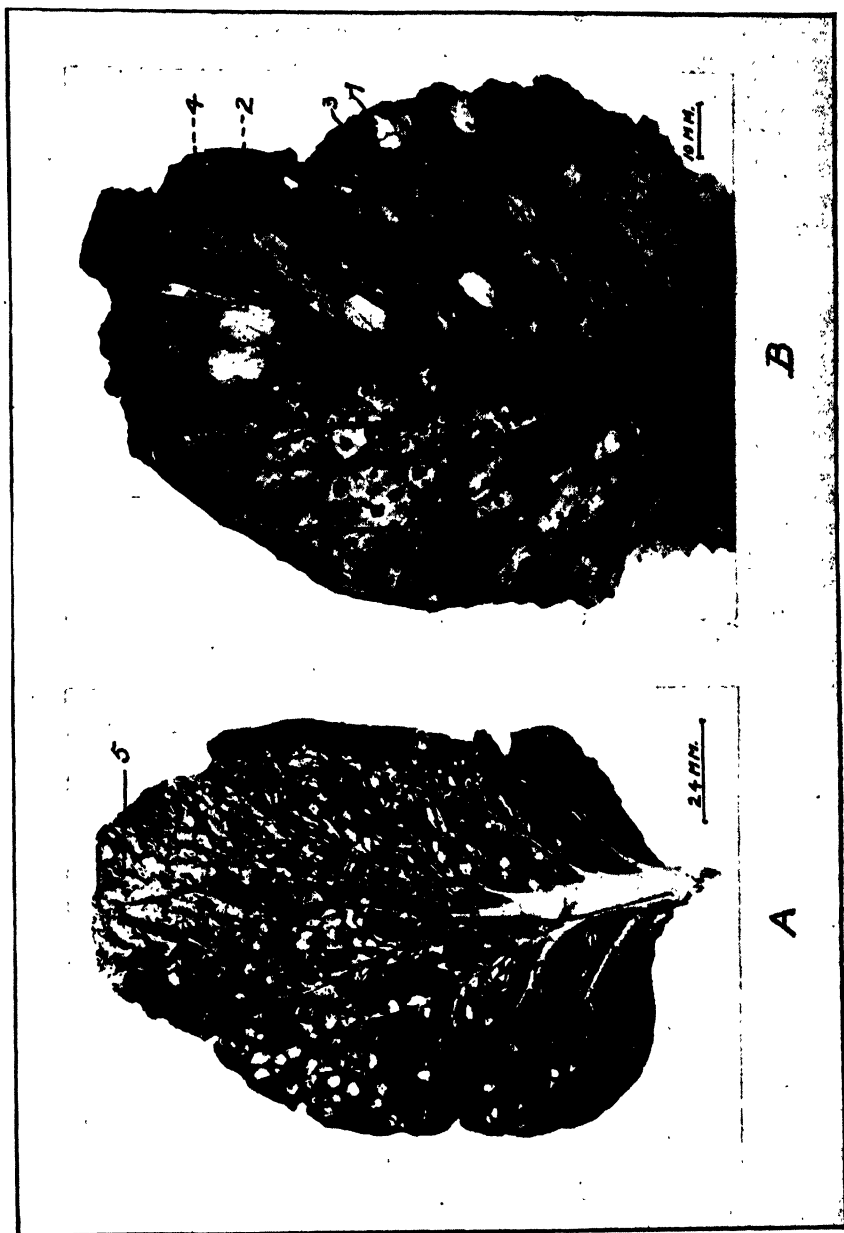
Photographed leaves of Chinese cabbage infected with *Cercospora albo-maculans*.

A. Numerous lesions which have coalesced at the tip of the leaf (No. 5).

B. A young, infected leaf.

No. 1-2. Large, orbicular lesions of paper-like appearance crossed by the dark brown veinlets. Pressed leaves sometimes retain dark green margins around the lesions (No. 3).

No. 3-4. Early symptoms.



PHYTOPATHOLOGICAL NOTES

Preliminary Report on the Gardenia Bud Drop.—One of the greatest trials of nurserymen who grow gardenias in quantity is the so-called “bud drop”—the falling off of buds shortly before flowering time. In the San Francisco Bay region, bud drop caused a loss of \$2,000 in one nursery alone in 1925.

The disease first becomes evident as a gradual discoloration of the pedicel and bud. The disease develops on the bud on the exterior of the calyx, where there are five pore-like structures, the extra-floral nectaries. This mode of entry of plant disease through extra-floral nectaries has not hitherto been described. The bright green color of the buds becomes dark cream, then yellow and finally dark brown. The bud gradually becomes moist and rotten and finally drops off in from four days to a month, the time depending on the temperature.

Thinking that the disease might be bacterial in nature, isolations were made on alfalfa agar from buds in various stages of infection. Within three days minute bacterial colonies were visible; two days later these were an inch in diameter, and all alike.

After pure cultures had been secured, many inoculation experiments were made. In one of the series, 20 healthy buds were inoculated. Most of these inoculations were made in the extra-floral nectaries of healthy buds. When leaves and stems were inoculated, the infection developed only when high temperatures prevailed.

In each experiment some of the buds were used as controls, but in every case only a few blossomed. The fact that a few controls did blossom shows that insect control and clean surroundings for the plants are important factors.

Careful observation in commercial greenhouses and under the controlled conditions of the laboratory established the fact that the disease was spread by mealy bugs and ants, which usually infest the beds in which the gardenias are grown. Experiments were repeatedly performed with insects taken from infected beds and with insects obtained from regions remote from any gardenia plants. Both mealy bugs and ants feed at the extra-floral nectaries, hence it is very probable that the disease may become widespread through these agents.

Temperature has a direct influence on the activity of the bacteria: higher temperatures are more favorable for their growth.

The bacteria causing the disease are rod-shaped, occurring singly for the most part, though occasionally two are joined endwise. Each bacterium

possesses approximately seven peritrichiate flagella. The organism therefore belongs to the group *Erwinia*. The colonies are smooth, circular in outline, of a yellowish hyaline mucose appearance, with a milky spot in the center of older colonies.—ALBERT WILSON, *Department of Botany, Stanford University, California*.

The American Type Culture Collection.—The recent publication of the first printed catalogue of the American Type Culture Collection of bacteria and fungi will awaken interest in this effort to preserve authentically-named viable cultures in a central place for distribution. Copies of the catalogue may be obtained from Dr. George H. Weaver, John McCormick Institute, 637 South Wood Street, Chicago. Cultures may be purchased from him at one dollar per culture, plus the cost of packing and postage. There are now about 200 carefully selected pure cultures of fungi in the collection. They are cared for by Mario Scandiffo, who works half time, under the supervision of Drs. Charles Thom and Margaret B. Church, of the United States Department of Agriculture, and is paid by the General Education Board and the Society of American Bacteriologists. Each culture is kept on a suitable medium in three places, representing different combinations of humidity with average temperatures of 0°, 7°, and 24° C. The cultures are transferred at intervals of 3, 6, and 12 months, depending on the organism and the temperature at which it is being maintained. As this arbitrary schedule is supplemented by continual supervision, few cultures have been lost.

Phytopathologists are urged to contribute cultures, both of newly described species and of strains of previously described species which they have studied. Each culture should be accompanied by as complete a history as possible and by references to the original description of the species and to special investigations.—ANONYMOUS.

Fifth International Botanical Congress, Cambridge, 1930.—At the International Congress of Plant Sciences (Fourth International Botanical Congress) held at Ithaca, New York, United States, in August, 1926, an invitation was conveyed from British botanists for the Fifth International Botanical Congress to be held in England in 1930. The invitation was accepted by the botanists assembled at Ithaca, and arrangements are now being made for the Congress to be held at Cambridge about the middle of August, 1930.

An Executive Committee has been formed to make arrangements for the Congress, consisting of Dr. F. F. Blackman, Professor V. H. Blackman, Dr. E. J. Butler, Professor Sir John Farmer, Professor F. E. Fritsch, Professor Dame Helen Gwynne-Vaughan, Dr. A. W. Hill, Professor W. Neilson Jones,

Sir David Prain, Dr. A. B. Rendle (treasurer), Professor A. C. Seward (chairman), Professor W. Stiles, and Professor A. G. Tansley.

It has been decided to organize the Congress in the following seven sections: morphology (including anatomy), palaeobotany, plant geography and ecology, taxonomy and nomenclature, genetics and cytology, physiology, and mycology and plant pathology.

Mr. F. T. Brooks, The Botany School, University of Cambridge, England, and Dr. T. F. Chipp, Royal Botanic Gardens, Kew, England, have been appointed honorary secretaries of the Congress, and any communications with regard to the Congress should be addressed to one or other of the secretaries.

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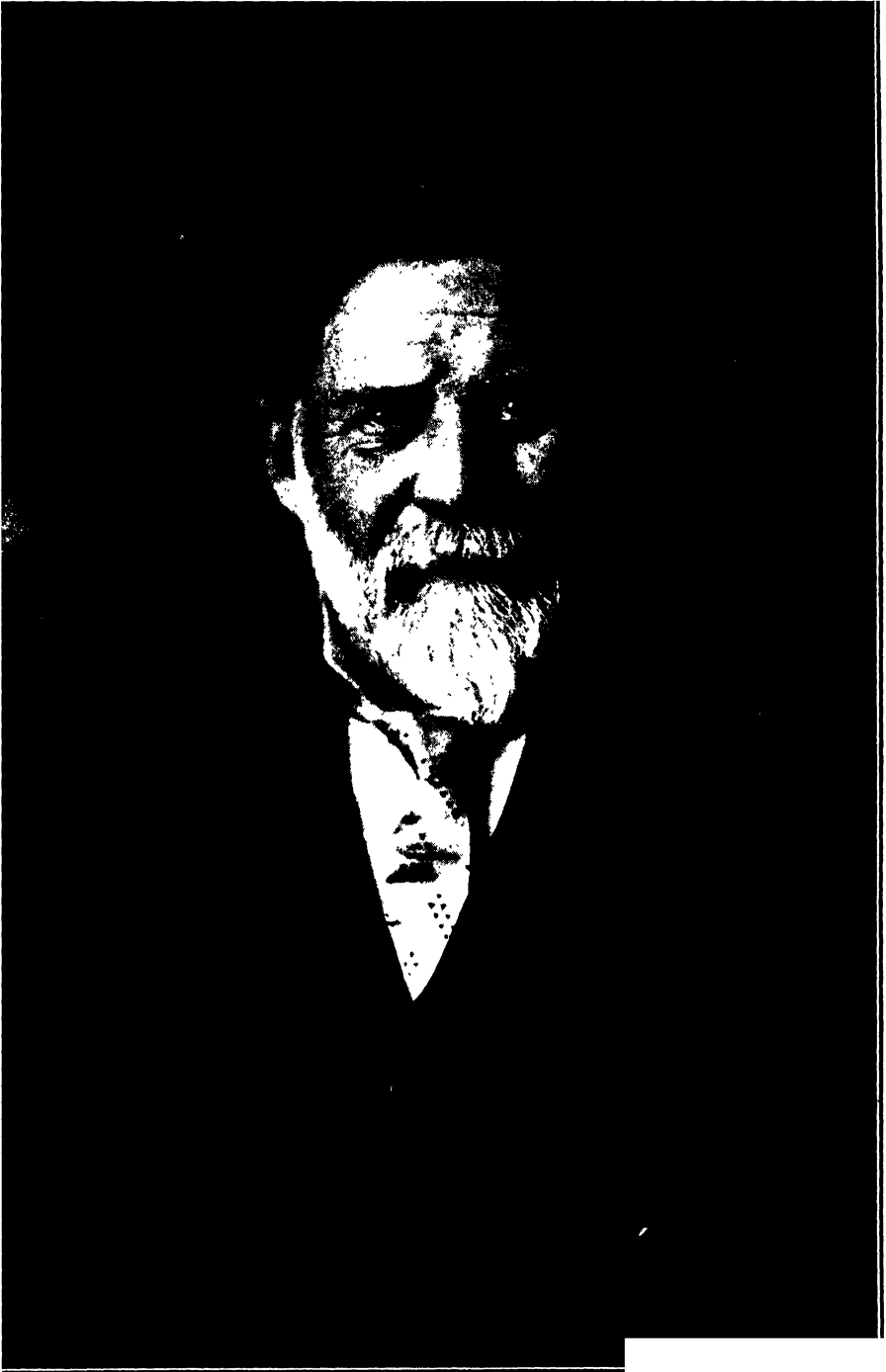
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ERWIN F. SMITH

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ERWIN F. SMITH

1854-1927

RODNEY H. TRUE

I suppose the purpose of a biographic sketch like this should be an attempt, while yet a man's life is vividly with us, to bring together such information concerning his efforts and achievements as have become a part of what we think and know and to recall once more those individual traits that came to be a part of the life experience of those who knew the deceased. To make such a sketch of most of us would be much simpler than to attempt to trace even the outlines of the very simple yet complex story of the years of Erwin F. Smith.

While the ground plan of his life was very simple, he developed the fundamental principle with much variation. The details were often elaborated, as his favorite Beethoven elaborated his simple themes, until a great variety seemed to appear in the pattern.

My acquaintance with our friend began at the Madison meeting of the American Association for the Advancement of Science, where I, as one of the younger men familiar with that charming region, was told off to pilot a party of botanists through the Dalles of the Wisconsin River. To my great good fortune Erwin F. Smith and Volney M. Spalding found places in my boat. As we drifted or rowed through that magic gorge, I was impressed by the close observation, eager attention, and complete appreciation shown by Dr. Smith toward everything about him, and the friendly familiarity then extended to me was never withdrawn during the succeeding years.

Thus when I was a student at Leipzig a few years later, I was not inattentive while attending Alfred Fischer's lectures in bacteriology to hear him mention an American named Smith who claimed that bacteria could grow in plants, that they did so grow and produced diseases in them as in animals. The naturally rather bitter tongue of Fischer denied this claim and laid Smith's "blunder" to a dirty technique in terms that an American present felt were intended to reflect rather broadly on the state of science in Smith's country. This was an episode in that most significant polemic

in which, like Pasteur in his day, Smith fought and fought strenuously for his glimpse of a very important truth. It gave me, who had winced as Fischer laid on the lash at Leipzig, a lively satisfaction to see Smith more than completely vindicated as work progressed.

That phase of phytopathology in which Dr. Smith gained his famous victory over Fischer and Robert Hartig was not the field of his maiden effort. Graduating from the University of Michigan in 1886, after taking a course in which biology received main emphasis, he promptly began a life of research by entering the United States Department of Agriculture in the fall of that year. He remained in this institution during the rest of his life. By some strange irony of events, better appreciated as the years have gone by, the young graduate was given the task of finding out the cause of peach yellows, a disease very destructive at that time in Delaware and Maryland. In spite of all the young investigator's enthusiasm and industry, the cause of the disease remained hidden. As he himself has written—in a summary of his work prepared in 1922 at the request of others—"After some years, I abandoned this research and devoted my time to other subjects, mainly, as I have often said, to save my reputation, but really because the problem appeared to me to be insoluble in our then state of knowledge. For that matter it has remained unsolved up to the present time."

One might speculate interestingly concerning the possible effect on Smith's later career and on the course of phytopathology in America had he attacked a less difficult problem first.

He next turned to the study of *Phytophthora infestans* and of *Plasmopara viticola*, but peach yellows, peach rosette, and later the "little peach" of Michigan and brown rot of peach were much on his mind and formed the subjects of important contributions prior to 1892.

He then turned away rather definitely from peach troubles and gave much attention from 1894 to 1910 to *Fusarium* injury. His work on this group of diseases on melons, cotton, cowpeas, tomatoes, potatoes, and cabbage is now a well-known chapter in American plant pathology. His paper on the "Fungous Infestation of Agricultural Soils in the United States" (1899) was the first paper on this subject published in the United States or elsewhere, insofar as it concerned *Fusarium* infections. This work on *Fusarium* opened the way to later work by Orton and others that resulted in the securing of highly resistant strains of most of the crop plants named.

Perhaps the greatest contribution that our country has made toward an understanding of plant disease lies in the field of bacteriology. Burrill's pioneer discovery in 1879-80 that bacteria were the cause of pear blight gave a glimpse into an unsuspected realm. It is not to be wondered at that this field, new and full of tempting obscurities, should have so powerfully

attracted the young researcher that he was able to write in 1922 that he had "done original researches on such diseases every year from that time to the present." He began his long series of brilliant and fundamental investigations with a study of cucumber wilt in 1893.

The immediately succeeding years were largely devoted to bacterial diseases. In 1897 Alfred Fischer brought out his "Vorlesungen über Bakterien," a book based on the lecture course that I had heard a few years before. In this book Fischer elaborated the attack on the work of Smith and other Americans and did it in a way that injected a rather bitter flavor into the controversy. This book brought a swift reply from Smith, who rightly felt himself called on to lead the defense. This he maintained with a thoroughness and persistence that led to the complete establishment of his claims.

I have heard Dr. Smith discuss this chapter in his scientific career in the quieter after years, and a tinge of regret always crept into his voice. That the truth should be gained by battle seemed at times still to be a necessity, something hardly to be regretted perhaps, but that the clouding of personal relations should follow was sometimes a sad price to pay. In a recent manuscript Dr. Smith refers briefly to this episode. He writes that "Fischer never forgave me, but I could not do otherwise; nor do I regret the polemic, since it cleared the air and advanced the science."

One is led to meditate a little concerning this controversy. It was intercontinental. American science had made an important discovery. Continental Europe had a habit of paying little attention to us. Pfeffer told me while I was a student at Leipzig that he did not have American botanical periodicals in the Institute, a partial file of the Botanical Gazette excepted, because there was little in them. He remarked that he never looked into them any way because he could employ his time more profitably otherwise. This was but a rather brutal way of saying truthfully that he thought little of American botany. I believe that same self-satisfied obliviousness was general. That America could originate anything new and worth while seemed to be out of the question, a possibility easily to be dismissed. When, therefore, stubborn insistence in the European periodicals, with a show of evidence, forced attention, irritation displaced obliviousness but, as scientists will eventually face facts—even those coming from an unexpected source—Smith finally won an increased measure of respect abroad for himself and for his country. It helped to break an opening through that semi-permeable wall of indifference that had always more or less effectually shut out the New World.

I shall not attempt to mention and comment on his long series of contributions to our knowledge of bacterial plant diseases. He had already

written three volumes of monumental proportions on this subject before his death and had three more to write. In 1922, he wrote that he hoped to finish volume IV, the manuscript of which was at that time well in hand. He never completed his great undertaking, but fortunately in 1920 he summarized much of this work in his well-known text-book, "Bacterial Diseases of Plants."

I cannot dismiss this feature of his work without mention of Dr. Smith's work on crown-gall and some of the consequences that he felt might come of it. His first acquaintance with this trouble began back in "peach-days," when in 1892-93 he sought some causal parasite for this lesion. "Bacteria," he writes, "were at that time not in mind," and as no constant features seemed to give a lead, the subject was for the time abandoned. In 1924, fresh from his work on olive tubercle, he returned to the study of overgrowths, specially that on the Paris daisy, this time with bacteria definitely in mind. He did not know till later that Cavara in Italy had already isolated a white organism from such overgrowths on grapes and had produced a few tumors with it. These crown-gall researches constituted an important item in his program after 1904. I shall not review the mass of detailed information, anatomical and otherwise, developed in this study. In his summary statement written in 1922, Dr. Smith indicated the gains made up to that point in a few sentences that may be quoted:

"We now know not only the morphology and biology of the organism causing the tumors but also that the type of the tumor varies with the part infected, that there are several strains of the organism and probably many, that isolations differ in virulence . . . that isolations are cross-inoculable to a very surprising degree . . . that some plants immune or nearly immune to certain strains respond vigorously to other strains and that some species are resistant to all strains so far as tested." He also outlines points unknown or needing further study.

In 1907, Dr. Smith conceived the idea from the involved microscopic structure of the tumor that its study might throw light on cancer, and "the more I have studied it the more analogies I have discovered," he wrote in 1922. His work on this phase of the problem brought him into active contact with others working on this scourge. That his contributions were appreciated by the medical fraternity is shown by his election to the presidency of the American Association for Cancer Research in 1925. He also received a certificate of honor in 1913 from the American Medical Association for his work on "Cancer in Plants." Many other evidences of similar tenor are not lacking.

I will not extend further this notice of his scientific work. Important matters have not been touched on, but the complete bibliography of his published work that follows will guide the reader to these.

It will also suggest the fact that Dr. Smith's interests were by no means summed up in his scientific activities.

The life work and character of Pasteur seem to have had a great fascination for him. This appears in several articles written about the great Frenchman and in numerous incidental references to him in his other work. His chief tribute, however, is seen in the translation of Emile Duclaux's "Pasteur: Histoire d'un Esprit," so appreciatively and faithfully rendered into English with the help of his assistant, Miss Florence Hedges.

I cannot close this sketch without further reference to those aspects of Dr. Smith's life that were developed outside of his laboratory. We are born with a rather complete circle of life possibilities that must inevitably be narrowed in order to give the intensity of effort demanded by our work. Thus we must early begin through neglect to kill off our possibilities one by one in order that a small part may be the more vigorous. Many of us resist more or less stoutly this forced atrophy and try to keep ourselves alive over the greatest possible part of our circumference. Others yield more readily. Dr. Smith strove with wonderful effectiveness to defend himself against the harmful results of specialization.

He developed a knowledge of French, German and Italian literature that opened to him worlds of intense pleasure. Often have I seen him pursue some theme from language to language with an enthusiasm and facility that showed how deeply he had read and thought. He read his Bible in a copy of the Vulgate; and Dante was a favorite with him, in Dante's own great language. Goethe was often quoted in the original. Seldom have I known a man, whatever his training and field of work, who brought such joy and understanding to the works of the great writers. His library was a sort of map of his mind. In it were all manner of noble things.

His ear never ceased to find delight in music more and more as the years went by, be it the music of the great poetry of the past or that poetry expressible only in mighty harmonies.

He took great delight in beautiful paintings, in sculpture, and in architecture. No road along which beauty might enter was blocked.

I think that while Dr. Smith was a true scientist to the very heart, he felt cramped by the physical world and sought greater freedom in the world of imagination where he could live as every man once in a while feels a desire to live. He returned from such escapes with sonnets or other spiritual treasure. These in part he collected and published in a limited edition that found its way to friends. In this privately printed volume, "Her Life and Mine," we see the acceptance of great sorrow and the utilization of it for the wider purposes of life.

It seems to me that Dr. Smith was fundamentally organized as artists rather than scientists are supposed to be organized. He was quick, enthusiastic, and strongly appealed to by beauty in all of its forms. I think that he may have had to learn the lesson of reserving judgment, of remaining skeptical, in short, the whole defensive attitude of science. Thus the imagination of the artist was fundamental, and by opening wide the book of nature it revealed to him the far reaches of life. Restraint and discipline made the poet into the scientist, whose great motto seemed to be to test all things, not once or twice, but many times before acceptance.

As a friend, he was quick and sympathetic, outspoken in praise and dispraise, loyal to the end.

Undoubtedly the future holds opportunities equal to those that he saw and grasped. Undoubtedly there are others who will accept them but not many can do as he did. Many of us will always miss our friend.

I am deeply indebted to Mrs. Smith and to Miss Florence Hedges, for some years associated with Dr. Smith in his laboratory and literary work, for access to material that has made possible much of what I have written. The portrait and the full bibliography that accompany his sketch are from the same source. Dr. F. V. Rand, associated for several years with Dr. Smith in his work, has also given me much appreciated help.

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1881

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1888

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The symbiosis of stock and graft. 615-621.

The action of light on bacteria. 671-674.

The role of calcium and magnesium. 674-676.

Woronin on *Sclerotinia*. 749-750.

Demonstration of photosyntax by bacteria. 750-752.

Detection of glukase by auxanographic methods. 752-753.

Fischer on bacteria. 847-851.

The mushroom gardens of South American ants. 851-854.

Root tubercles of leguminosae. 898-903.

Bactericidal action of metals. 933-936.

Saccardo's color scale. 1009-1010.

Kroeber's transpiration experiments. 1010.

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Germination of refractory spores. 64-65.

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A second Rafinesque. 321-323.

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Whitney on Florida. 602.

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THE THREAD BLIGHT DISEASE CAUSED BY CORTICIUM KOLEROGA (COOKE) HÖHN., ON CITRUS AND POMACEOUS PLANTS

FREDERICK A. WOLF AND WALTER J. BACH

The name "thread blight" was first applied to a disease of tea in Northern India. It has come, however, to be employed as a designation for those diseases of various species of trees and shrubs which manifest themselves by conspicuous white to dark brown strands of fungous hyphae on the leaves, twigs, and smaller branches. Thread blights are of common occurrence in the tropics but are so rare in temperate countries as to have escaped the notice of many well-trained and experienced plant pathologists.

The attention of the staff of the United States Department of Agriculture Citrus Disease Field Laboratory, Orlando, Florida,¹ was directed in July, 1920, to a peculiar disease on grapefruits (*Citrus grandis*) and oranges (*Citrus sinensis*), which came to be known locally as "the shoe-string disease." This malady was first observed in the vicinity of Okeechobee, Fla., and had undoubtedly existed there for several years but had not previously been reported from the United States. The grove in which it was first noted is situated in a low hammock from which the towering cabbage palmettoes (*Sabal palmetto*) had not been removed prior to planting with *Citrus*, and is surrounded by a very dense forest. In due time a report (22) of the occurrence of this disease was prepared in which the causative fungus was tentatively identified as *Corticium stevensii* Burt. Positive identification has been impossible, however, until the past summer (1926), when the fungus was observed in the fruiting or basidial stage. In consequence of the finding of sporophores, a study of the morphology of the fungus was begun. It was apparent as the work progressed that the Citrus thread blight fungus is identical with hypochose of apples and pears.

A cooperative undertaking, upon which no report has been prepared, was begun in 1923 for an investigation of hypochose of pomaceous fruits.² In the light of this fact and of the results of the preliminary studies on the Citrus thread blight fungus, it was deemed advisable to modify the plans

¹ Field observations and control experiments, extending over three seasons, were made by J. R. Winston, formerly of this laboratory.

² It has been impossible to carry to completion this cooperative study as originally planned by Professor H. H. Whetzel, Cornell University, Ithaca, N. Y., and the senior writer. Special thanks are due Professor Whetzel, however, for his critical reading of this manuscript and for his suggestions.

so as to make a comparative study of the diseases on both Citrus and pomaceous plants and of the morphology and taxonomy of the thread blight fungi from both types of plants, and then to assemble the results in one report. The results of these studies are herein recorded.



FIG. 1. Affected grapefruit leaves. The dead leaf below is attached to the green one by a hyphal mat. Rhizomorphs stripped from the twigs and attached to the petioles are shown at the center. The upper leaf bears a sporophore of *Corticium koleroga*.

HISTORY OF THE DISEASE AND HOSTS

Little attention appears to have been given by investigators to the thread blights of *Citrus*. A review of published reports indicates that at least two distinct species of *Corticium* are involved. One, *C. salmonicolor* B. and Br., mentioned by Stevenson (20), occurs not only on various species of *Citrus* and its relatives but on a wide variety of other woody plants as well. It forms pinkish sporophores upon the surface of the bark and is designated "the pink disease" throughout the tropics. The other, *C. koleroga* (Cooke) v. Höhn., is the cause of the well known "koleroga" disease of coffee (*Coffea arabica*) in Porto Rico, Jamaica, Cuba, the Lesser Antilles, Trinidad, Surinam, Venezuela, Colombia, Guatemala, Brazil, India, Java, Malaya, Queensland, and Congo, as indicated by the investigations of Stevenson (20), Cooke (4, 5, 6), Kuijper (11), Fawcett (8, 9, 10), Burt (1, 2), Stevens (18), Coleman, Venkata Rao and Narasimhan (3) and Nowell (13). The last named writer reports the presence of the same parasite on sour orange (*Citrus aurantium*), *Hibiscus*, *Croton*, *Luffa*, *Cucumis*, and *Codiaeum*. Additional hosts listed by Stevenson (20) include the mango-steen (*Garcinia mangostana*), the rubber tree (*Hevea brasiliensis*), and certain species of *Citrus*. Coleman and his associates (3) list as hosts in India: *Jasminum* sp., *Randia dumentorum*, *Tabernaemontana coronaria*, *Gardenia gummifera*, *Wendlandia notoniana*, *Canthium parviflorum*, and *Pavetta* sp.

The fungus as it occurs on pomaceous hosts in the United States has been made known through a preliminary report by Stevens (17) in 1907, which was followed, two years later, by the detailed investigations of Stevens and Hall (19). They designated it *Hypochnus ochroleucus*, a name which had been given by Noack (12) to a fungus on apple and quince which he had under observation in Brazil in 1898. Their list of hosts in North Carolina include apple, pear, quince, snowball (*Viburnum* sp.), and lilac (*Syringa vulgaris*). Burt (1 and 2) has added to this list *Codiaeum variegatum* collected in Trinidad, and Nowell (13) regards the thread blight of cacao (*Theobroma cacao*) and nutmeg (*Myristica fragrans*) in the Lesser Antilles as due to the same fungus. The same fungus,³ to all appearances, under the name *Sclerotium pruni spinosa*, var. *ramicola*, has been collected on Mariana plum in Louisiana.

The writers' field observations on thread blight are confined to its presence on apple and pear in North Carolina and on pear, grapefruit, sweet orange, pecan (*Hicoria pecan*), and pomegranate (*Punica granatum*) in Florida. Collections⁴ in the Department of Plant Pathology, University

³ Specimens for examination were supplied from the herbarium of the New York Botanical Garden through the kindness of Dr. F. J. Seaver.

⁴ These data were supplied through the courtesy of Erdman West.

of Florida, Gainesville, Fla., show that in addition it has been found in Florida to attack tung oil (*Aleurites fordii*), tallow tree (*Sapindus utilis*), persimmon (*Diospyros virginiana*), pistachio (*Pistacia chinensis*), fig (*Ficus carica*), Virginia creeper (*Ampelopsis quinquefolia*), sour orange, and rose (*Rosa sp.*).



FIG. 2. Thread blight on grapefruit showing mycelial strands, paucity of sclerotia, defoliation by breaking at the petiole and suspension of the curled, dead leaves and petioles by rhizomorphs.

OCCURRENCE OF THREAD BLIGHT ON CITRUS IN FLORIDA

The known occurrence of thread blight on *Citrus* in Florida is confined to four adjoining groves in the vicinity of Okeechobee.⁵ It does not occur upon all of the trees in these groves nor is it confined to groups of trees as would be expected if the spread of infection was outward from separate foci. Instead diseased trees occur here and there throughout the grove. They can be readily detected at any time during the entire season when *Corticium* is active. This period corresponds with the rainy season which normally extends from the middle of June until the latter part of September. During the remainder of the year the fungus remains dormant and can be noticed only upon rather close inspection. Leaves, twigs, larger limbs, and fruits are involved.

APPEARANCE OF THREAD BLIGHT

On Citrus.—Attention is first directed to the disease by the presence of groups of blighted leaves promiscuously disposed throughout the tree. Those most recently killed are dry and curled and still attached. Older ones are broken off at the petiole and dangle, being suspended by mycelial threads. Closer examination shows that newly attacked leaves are still green, with whitish, powdery patches covering a portion of or the entire lower leaf surface. These patches are the fructifications of the fungus and consist of delicate, arachnoid, fungous membranes. Leaves in more advanced stages of disease exhibit large, indefinitely margined, dead areas, whose lower surfaces are covered by a brownish hyphal web. Affected leaves which are in contact may become matted together, being bound by mycelium (Fig. 1), and when they become detached from the petiole will remain dangling by brown rhizomorphs (Fig. 2).

The rhizomorphs can readily be traced from the sporophores backward along the petioles to the twigs and thence to the older wood. They course

⁵ On June 28, 1927, the senior writer observed thread blight on grapefruit in a grove of approximately 100 acres located near Deep Lake, about ten miles north of Everglades, Florida. This grove is isolated by a distance of about 25 miles from the nearest planting of Citrus. The portion of the Everglades in which it is situated consists of immense swamps which surround islands of dense forest. In the section surrounding Deep Lake outcrops of coquina rock appear at the surface.

It has not been possible to determine how long the disease has been present in this grove, although in all probability it has existed there for a term of years. The information in hand as to the source of the Citrus trees and the possibility of the presence of *Corticium koleroga* on the young trees indicates that they were free from the disease when they were transplanted.

Thread blight had evidently begun to spread in the grove with the advent of the rainy season, which occurred about two weeks prior to June 28. Basidia with basidiospores were present in abundance on the newly attacked leaves in collections made on this date.

along the lower side of the twigs or on the portions least exposed to the direct rays of the sun. Chestnut brown sclerotia are sparsely present on the twigs of the current year and on those of the previous season (Fig. 2). Those on older wood are darker and manifestly desiccated and exhausted. Affected twigs die back as the result of defoliation by thread blight.

The attack on the fruit follows the extension of the mycelial strands along the fruit spurs, and the fungus appears as a conspicuous, fibrillose, anastomosing meshwork over the surface of the young fruit (Fig. 3). These strands eventually spread out and toward their extremity are separated into simple hyphal threads. The areas beneath these distal portions assume a scalded appearance owing to the death and collapse of the rind tissues (Fig. 4).

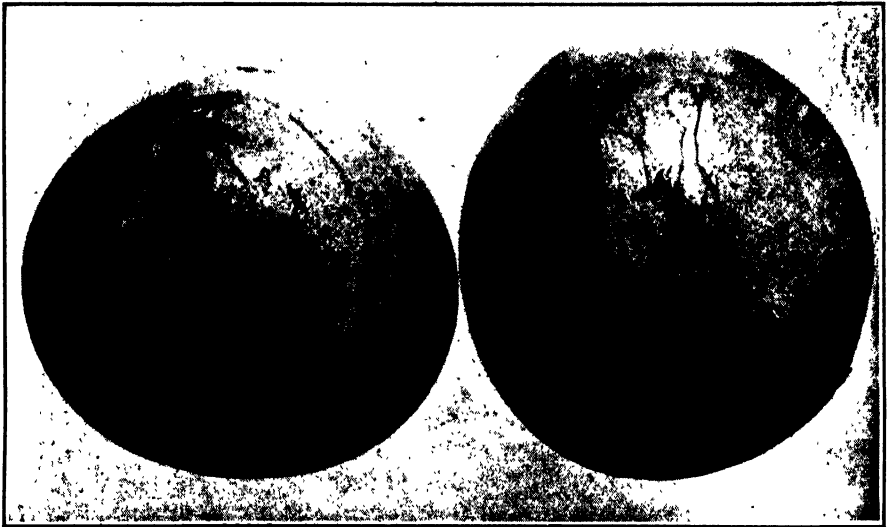


FIG. 3. Young grapefruits attacked by *Corticium koleroga*. A network of strands occur on the fruit at the right; the other one shows the initiation of the "scald" stage.

On Apple and Pear.—The writers' observations on the appearance of thread blight on apples and pears confirm in all essential features the account given by Stevens and Hall (19). At first glance affected trees simulate the appearance of fireblight in that dead leaves throughout the tree remain hanging. Closer inspection, however, of dead and dying leaves reveals the dense, brown, fungous membrane upon the lower leaf surface. This membrane is much more prominent than on *Citrus* and may be stripped off intact. On newly infected leaves which are still green this membrane

is very delicate and white. Before it has developed sufficiently to cover the entire leaf, basidia are formed in abundance, especially along the margin of the leaf (Fig. 5). These fructifications are the terminations of rhizomorphs, at first white and then brown, which extend outward along the petioles from the sclerotia on the twigs (Figs. 5 and 6). These sclerotia are hemispheric, chestnut brown bodies which are formed abundantly upon the twigs of the current year. Those on older wood are darker in color and appear dead. They may remain viable, however, until the second season after their formation. As attested by the observations of L. A. Ammon,⁶ County Agent, Brevard, North Carolina, the sclerotia which were formed during 1924 remained dormant during the summer of 1925 because of the drought in western North Carolina. With the advent of abundant



FIG. 4. Grapefruits with large "scalded" areas; mature lesions on young fruits.

rainfall late in July, 1926, dormancy was broken and the fungus spread rapidly.

The thread blight fungus does not appear to cause the death of apple and pear twigs, but trees in which the infection has persisted for years are stunted as evidenced by the abundance of short twigs.

⁶ Special thanks are extended to L. A. Ammon for shipments of specimens and for the use of notes bearing on his observations on this disease.

Stevens and Hall (19) have reported that sclerotia are less frequently present on apple fruits than on twigs. This appears to be the case with all varieties observed except Russets. The entire surface of Russets may be abundantly covered with sclerotia (Fig. 7); whereas occasional fruits of other varieties bear sclerotia in the region of the blossom end or stem end only. Strands which originate from the sclerotia on twigs migrate along the fruit spurs, and thence to the fruit. Infection of the fruit follows. The organism does not appear to be the direct cause of decay of the fruits but affected fruits are rather quickly destroyed after harvesting by the common storage molds.

On Pecans.—Thread blight on pecan has been collected in the vicinity of Okeechobee, Fla., in a small group of trees about 20 years of age which are growing along the edge of a Citrus grove. The character of the disease on this host as far as size, number and distribution of sclerotia on the twigs (Fig. 8) are concerned, is entirely similar to that on apple and pear. The appearance on the foliage is somewhat different, however, in that the prominent strands which extend along the petiole from sclerotia of the previous season become very small and inconspicuous as they course outward on the leaflets and attack them progressively from the base of the leaf upward. The affected leaflets are shed in turn, beginning with the lowermost, but they may remain suspended for a time by the rhizomorphs. Membranous mycelial wefts of the consistency of those on *Citrus* are formed on the lower leaf surface. The leaf tissues involved become brown and dry, which results in the distortion of the leaves.

On Pomegranate.—The disease on pomegranate differs in appearance in several respects from that presented by pomaceous and Citrus hosts. The sclerotia on twigs are as numerous as on apples and pears, but are small, irregular in outline, and crustose (Fig. 9). The rhizomorphs commonly have ribbon-like lateral expansions. The entire lower leaf surface is covered by a very dense, tawny, fungous membrane which may be torn off in frayed strips as shown in figure 9. This membrane is considerably thicker than on any other species which have been observed.

ISOLATION OF THE FUNGUS

No account has come to hand, except that of Coleman and his coworkers. (3), of the isolation and cultivation of the thread blight fungus in pure culture. Repeated unsuccessful attempts have been made to isolate it by planting sclerotia, and fragments of rhizomorphs and of mycelial wefts on agar. Either no growth at all has been secured by this method, or the growth was quickly overrun by species of *Colletotrichum* and *Pestalozzia*, which are veritable fungous weeds in Florida. Accordingly, efforts were

directed toward securing more favorable material for the isolation trials. It was found in the preliminary tests that the fungus from affected tung oil twigs could be induced to grow when placed in contact with twigs of living grapefruit trees. On June 13, tung oil twigs⁷ bearing an abundance of



FIG. 5. Rhizomorphs, sclerotia and sporophores of *Corticium koleroga* on pear.

⁷ This material was supplied through the kindness of Erdman West.

sclerotia, which had been collected April 27, were tied to young twigs of pears, grapefruits, and oranges. They were then wrapped with moist absorbent cotton and the tips of the inoculated twigs encased in paraffin paper. After seven days young rhizomorphs had developed and were found to have extended for an inch or more over the surface of all inoculated plants. Two days later, delicate white wefts had formed on the lower leaf surfaces, which upon microscopic examination proved to be sporophores. Leaves which bore fructifications were taken from each species of inoculated plant and attached, with the lower surface directed down, within the covers of poured agar plates. This permitted the basidiospores on ejection to lodge on the surface of the agar below. Within 12 hours an abundance of spores had fallen. They germinated promptly (Fig. 10), and pure cultures were obtained in these plates when reasonable care was taken to prevent contamination. Transfers were then made to various substrata by cutting out blocks of agar containing germinated basidiospores. The resultant pure cultures from this series were designated, because of their origin, as the

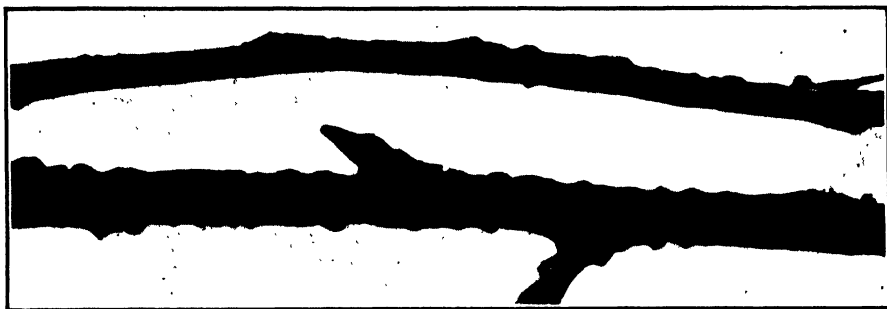


FIG. 6. Thread blight on apple twigs showing sclerotia and rhizomorphs.

tung oil thread blight fungus, a designation which was employed to identify it in the comparative cultural studies. This method of isolation is essentially like that used by Coleman and his assistants (3), who obtained pure cultures from basidiospores of this fungus from coffee.

During July, 1926, collections of thread blight in fruiting condition were made from grapefruit, pear, pomegranate, and pecan. Isolations from all except pomegranate were made by trapping the fallen basidiospores, according to the method which has just been described for isolating the tung oil fungus.

In a few cases isolations have also been effected by planting bits of recently infected leaves on agar plates. The surface of the leaves used was disinfected by immersion in alcohol; the alcohol was then removed by flaming. This method has little to commend it in isolating the thread

blight pathogene because various secondary invaders follow infection by the thread blight organism very closely and, in culture, grow out from the diseased tissues much more rapidly than the pathogene. Furthermore, the planting of old sclerotia has not proved to be a favorable means of isolating the fungus, although pure cultures have been obtained from young sclerotia.

In the comparative cultural studies use has been made of potato dextrose agar, cornmeal agar, sterilized pigeon pea stems (*Cajanus indicus*), steamed potato cylinders, and sterilized slices of immature grapefruits.

Growth on all these substrata is sufficiently rapid to form colonies of loose white mycelium within a week. They then become tinged, and at maturity are fuscous, resembling closely in color old mycelial wefts on the foliage. With the exception of a culture isolated in 1924 from sclerotia on apple twigs, no evidences of sclerotia have appeared in any of the cultures on any of these media.⁸ None of these cultures have developed sporophores. Further, no discernible differences in culture have been noted between thread blight from tung oil, grapefruit, pear, and pecan.

PATHOGENICITY

The writers' first efforts to secure experimental proof of the pathogenicity of the thread blight organism were made in attempts to isolate it, as has been recounted on previous pages. Subsequently several series of crude inoculations were made. On July 1, infected twigs of pecan and of grapefruit were used to inoculate pear and grapefruit. They were applied to the twigs, wrapped with wet absorbent cotton and enclosed in paraffin paper to preserve a high relative humidity. By July 8, infections had taken place and sporophores had formed both on pear and on grapefruit from the inocula from both sources. Isolations were then made by suspending some of the infected pear and grapefruit leaves over agar plates. Pure cultures of *Corticium* were secured from basidiospores which fell and germinated on the surface of the agar.

On July 16 another series of crude inoculations on pears and grapefruits was made. In this trial, affected twigs of pear and pomegranate, which had been collected at Gainesville, Fla., a few days previously, were employed as inocula in the same manner. After eight days conspicuous sporophores had developed on each inoculated plant. By this same method of isolation pure cultures of the thread blight fungus were secured from leaves of both species artificially inoculated.

In summary, the results of the tests on pathogenicity show that infection of both pear and grapefruit readily follows the use of a crude method of inoculation of the organism from tung oil, pecan, pomegranate, pear,

⁸ An abundance of sclerotia have appeared in cultures of the fungus isolated from grapefruit on June 28, 1927.

and grapefruit. It is reasonable to suppose, therefore, that reciprocal infections of any of these species would result from inoculation with the thread blight organism from any one of the others. It has not been possible to make such trials, however, because of the lack of suitable plants for use in inoculation.

Inoculations with pure cultures have been limited to the use of cultures originally isolated from tung oil, pear, and grapefruit. The inoculum was applied by cutting out bits of colonies on agar, and binding them with wet absorbent cotton on twigs of pear and grapefruit. The tips of inoculated twigs were then wrapped with paraffin paper to conserve moisture. Infections were invariably evident in the course of a week. Basidia with spores developed in due time, and after three weeks mature sclerotia had formed. The studies on pathogenicity with pure cultures of the fungus, while limited in number, confirm entirely the results of the crude inoculations.

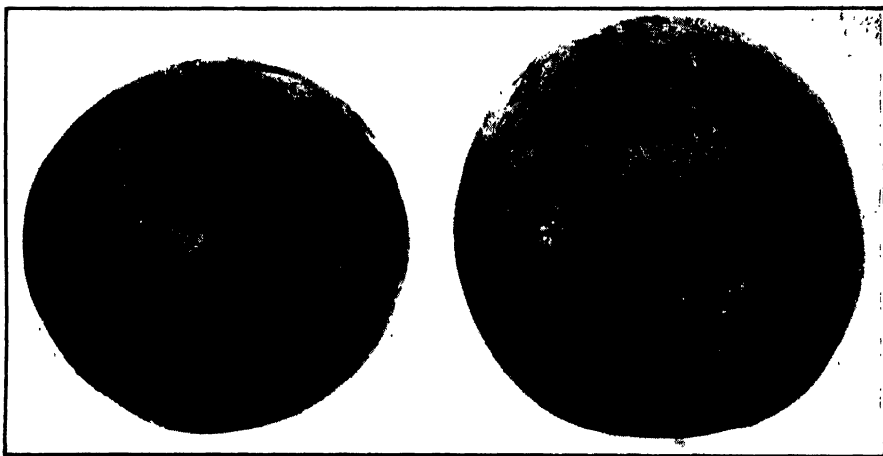


FIG. 7. Mycelial strands and sclerotia in abundance on Russet apples.

MORPHOLOGY OF THE FUNGUS

Consideration was given to this phase of the problem to determine whether a comparison of mycelia, sclerotia, basidia, and basidiospores both from natural infections on pear, grapefruit, pecan, and pomegranate, and from artificial inoculations on pear and grapefruit would substantiate the conclusion that the thread blight disease on the various hosts is caused by one and the same fungus.

When microscopic preparations are made by mounting hyphae from fresh material of any of the plants mentioned above, they contain rather coarse elements, hyaline or slightly colored, 4.5–7 μ in diameter, which are plainly *Rhizoctonia*-like in method of branching and septation. No clamp

connections have been noted. The cells which comprise the sclerotia and the intercellular mycelium are binucleate (Pl. XXVI, D), a condition which is of common occurrence among Basidiomycetes. The hyphae from different sources are indistinguishable, one from the other, as shown by figure 10: A, B, and E represent hyphae from natural infection on pear, apple and grapefruit; C represents hyphae from pear artificially inoculated with the tung oil fungus, and D represents hyphae from grapefruit inoculated with the fungus from pecan. Furthermore, no discernible differences can be noted between hyphae from cultures and those from parasitized leaves and twigs.

Sclerotia from apples and grapefruits were embedded in paraffin, sectioned, and stained. These sections show that the sclerotia are always seated in clefts or crevices and never arise on smooth surfaces (Pl. XXVI, G and H). The necessity for such places of attachment for sclerotia accounts no doubt for their occurrence in abundance on the fruit of Russet apples, since their entire surface is covered with short fissures which are arranged concentrically about the stem. All other varieties of apple are known to lack these crevices or to possess them only at the blossom and stem ends.

A cross-section through a sclerotium on Russet fruits (Pl. XXVI, F) shows that it consists of brown, thin-walled cells, which typically constitute a uniform fungous parenchyma (Pl. XXVI, I). Intercellular spaces may be present, and in some the central medullary portions consist of rather loose hyphal elements (Pl. XXVI, J) such as occur in the sclerotia of *Corticium vagum* (24). There is no evidence of a differentiated cortical layer such as occurs in the sclerotia of the genus *Sclerotinia*. The outer portion is to be regarded as a pseudorind, the cells of which in mature sclerotia are more or less collapsed (24), resulting perhaps from desiccation.

The expanded basal portion which serves as an anchor or holdfast rests closely upon the pulp cells of the fruit or, as the case may be, upon the corky layer of the outer bark of the twigs. These sections show no evidence of extension of hyphae from the holdfast into the subjacent tissues. If there is any withdrawal of food material from these host tissues by the holdfast it is not manifest by a collapse of the cells. The scarcity of fissures in the bark of grapefruit twigs undoubtedly accounts for the paucity of sclerotia on this host. Those which do occur, when sectioned, as shown in Plate XXVI, B and K, are indistinguishable in structure from those on apple fruits and twigs. They are secured by holdfasts, and the tissues upon which they are closely seated appear to be normal in every respect (Pl. XXVI, K).

An examination of microscopic preparations of newly formed basidia shows that they arise as terminations of short lateral branches. They are simple, ovoid, 10–12 by 7–8 μ with four, rarely six, slender sterigmata. The

basidiospores are hyaline, flattened on the opposed faces, rounded above and tapered below, 9–13 by 3.5–5 μ , with 10.5 by 4.5 μ as the most common size. There are no detectable differences between basidia and basidiospores on the several host species as is apparent from figure 10, A and B, which represent these structures from natural infections on pear and grapefruit respectively; and C and D, which represent artificial inoculations of the tung oil fungus on pear and of the pecan fungus on grapefruit respectively. This accords entirely with the observations of Burt (1), who states that the “microscopic characters of *C. stevensii* and *C. koleroga* are within the limits of fluctuation of a single species.”



FIG. 8. Pecan twigs and petioles bearing mycelial strands and sclerotia.

PATHOLOGICAL ANATOMY

Burt (1) sectioned leaves bearing sporophores of *C. stevensii* but secured no evidence of intercellular hyphae. Neither was any evidence of penetration by *C. koleroga* found in leaves of coffee by Coleman and his co-workers (3).

Lesions on leaves of apple, pear, grapefruit, pecan, and pomegranate, and tissues of affected fruits of apple and grapefruit were sectioned in paraffin and stained with Haidenhains iron-alum haematoxylin to determine the relationship of the pathogene to the tissues. Plate XXVI, A, C, E and L, represent vertical sections of diseased leaves of grapefruit, pear, pomegranate and pecan respectively. The fungus manifestly gains entrance in all cases through the stomates and courses between the cells, involving all of the tissues.

Since no vegetative hyphae have been found in the host tissues at the base of the sclerotia, as has been indicated previously, it is entirely probable that the food necessary for sclerotial formation is taken from the leaf tissues

by the intercellular mycelium and translocated to them. At any rate the host cells of mature lesions are collapsed, and the protoplasts are shrunk and stain more deeply.

The rind tissues of diseased grapefruits are affected in the same manner as the leaf tissues. Plate XXVI, M, shows the intercellular mycelium within the tissues of the outer rind.

The present observations show the same type of relationship of pathogene to host in the case of all host species examined.



FIG. 9. Small, crustose sclerotia on pomegranate twigs with the dense, fungous membrane partially stripped off of the leaf in the upper left corner.

ETIOLOGY

The cause of thread blight is a basidiomycetous fungus, *Corticium koleroga* (Cooke) v. Höhn. This organism was first described in 1876 by Cooke (4 and 5) from collections on coffee sent from Mysore, India. He regarded it as a Hyphomycete and designated it *Pellicularia koleroga*. Two years later, Ernst (7), in his investigations of the "candelillo" disease of coffee in Venezuela, described this fungus as a powdery mildew, *Erysiphe scandens*. Fawcett (10), in 1915, compared the coffee blight fungus in Porto Rico with specimens sent from India by Dr. E. J. Butler and found them to be identical. It appears, however, from his figures of holdfast cells that he had under observation young basidia but failed to recognize the basidiomycetous nature of the pathogene.

Von Höhnelt (23), in 1910, redescribed the fungus from Cooke's type and assigned to it the name *Corticium koleroga*.

The thread blight fungus on apples, pears and quinces in the southern United States was identified in 1907 by Stevens (17) as *Hypochnus ochroleucus*. Noack (12, p. 80) first noted it in Brazil as a parasite on pomaceous plants, in 1898, and named it *Hypochnopsis ochroleuca*, which was changed

in Saccardo's *Sylloge Fungorum* to *Hypochnus ochroleucus*. Burt (1) recognized that this fungus should properly be regarded as a *Corticium*, which differs, as he concluded, from *C. koleroga* by possessing sclerotia, and thicker, darker-colored fructifications. The supply of material upon which his observations were based was of necessity limited, however, to herbarium specimens, only two collections of which were in fruiting condition. He therefore proposed the new name *C. stevensii*, as there was already a valid *C. ochroleucum*, a binomial which had been employed by Bresadol in 1892.

The results of the present studies, which are based on the similarity of

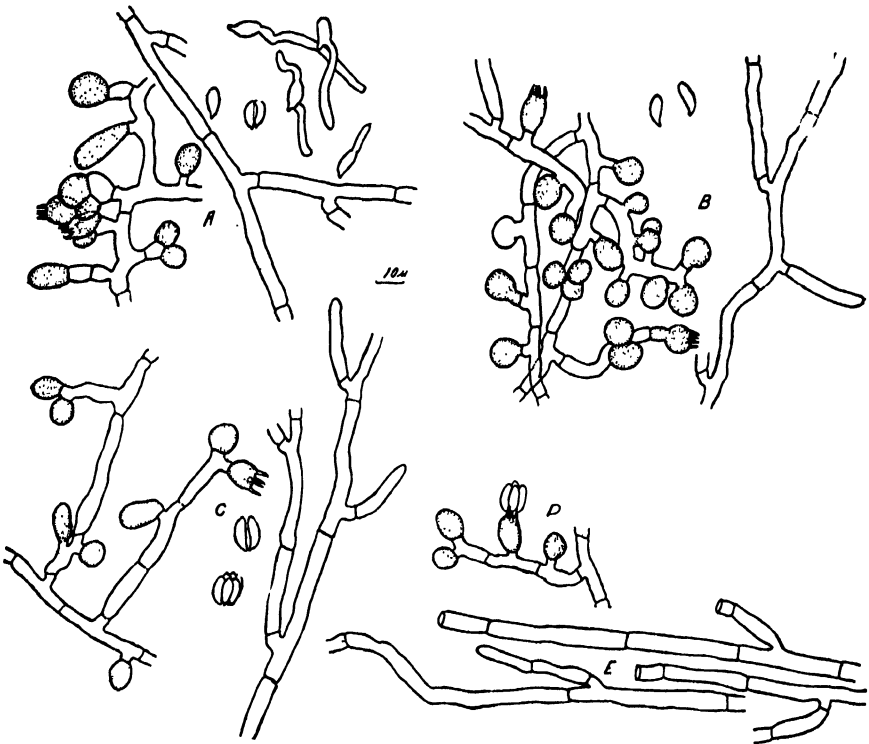


FIG. 10. A. Hyphae, basidia, basidiospores, and the germination of basidiospores of the thread blight fungus on pear, natural infection. B. Hyphae, basidia and basidiospores on grapefruit, natural infection. C. Hyphae, basidia and basidiospores of *Corticium koleroga* from pear inoculated with the tung oil fungus. D. Hyphae, basidia and basidiospores from grapefruit inoculated with the fungus from pecan. E. Mycelium of *C. koleroga* from apple fruit.

morphological and cultural characters of the organism from several host species, and on its ability to infect both pomaceous and Citrus plants, show that *C. koleroga* and *C. stevensii* are identical. The following synonymy therefore applies to this thread blight fungus.

Corticium koleroga (Cooke) v. Höhn. Sitzungsber. K. Akad. Wiss. Wien. 119: 395. 1910.

Pellicularia koleroga Cooke, Grevillea 4: 116. 1876; Pop. Sci. Rev. 15: 164. pl. 135. fig. A-C. 1876; Linn. Soc. Bot. Jour. 18: 461. 1881; Sacc. Syll. Fung. 4: 149. 1886.

Erysiphe scandens Ernst, Estudios sobre las Deformaciones, Enfermedades, y Enemigos del Arbol de Café in Venezuela, p. 16, fig. 5. 1878.

Hypochnopsis ochroleuca Noack, Boletim do Instituto Agronomico do estado de Sao Paulo em Campinas 9: 80. 1898.

Hypochnus ochroleucus Noack, Sacc. Syll. Fung. 16: 197. 1902.

Corticium stevensii Burt. Ann. Mo. Bot. Garden 5: 119-132. 1918.

GENERAL CONSIDERATIONS

It is a matter of considerable interest that the fungus under consideration is adapted to utilize such a wide variety of host species and is paralleled in this respect by the well-known *Corticium vagum*. The records of collections, as has been previously indicated, show that near Okeechobee, Fla., pecan, grapefruit, sweet orange and sour orange trees which are in adjacent rows are attacked; while at Gainesville, Fla., the fungus has been collected on tung oil, tallow tree, fig, pistachio, persimmon, and pomegranate, all of which were growing in close proximity. In Mysore, India, the fungus has been observed to have spread from leaves of coffee to leaves of *Jasminum* sp. in contact with them and also to have attacked various other species which were growing near (3). In the West Indies it has been collected, as previously stated, on coffee, *Citrus*, and many other species. It therefore is reasonable to assume, as the thread blight fungus from *Citrus* can attack various other plants, including Pomaceae, and the thread blight fungus from pomaceous species can infect *Citrus* and in addition several widely different species, and as the fungi from *Citrus* and pomaceous plants cannot be distinguished morphologically, that they are one and the same. The observations by various investigators on range of hosts, when interpreted in the light of the writers' infection experiments, indicate that in all probability all of the host species which have been enumerated in a previous paragraph are parasitized by one and the same species of thread blight fungus. This organism, in the light of these facts and on the basis of proper usage, should be regarded, therefore, as *Corticium koleroga*.

The most interesting points of contrast between *C. koleroga* and *C. vagum*, when it is recalled that both hibernate by means of sclerotia and that both have a Rhizoctonia vegetative stage and a basidial stage of the *Corticium* type, are that the former is confined to woody plants and is aerial

in habitat while the latter is confined to herbaceous plants or to seedlings which have not yet become woody, and is soil harbored.

All of the evidence in hand indicates that the sclerotia of *C. koleroga* are of primary importance in perpetuating the fungus from season to season. Once the fungus has become established on a twig or branch, it spreads by means of rhizomorphs and thus involves the new growth of each succeeding season, while at the same time the older sclerotia and rhizomorphs on the older twigs perish and are weathered away. The basidiospores which are formed during summer serve to initiate new infections on trees already diseased and, in all likelihood, are disseminated by rains, heavy dews and air currents.

This thread blight appears to be restricted to rather limited localities in any given area because of the requirement of high humidity and high temperature. This generalization is in accord with the observations of Noack (12), Stevens and Hall (19), Fawcett (9 and 10), Coleman et al. (3) and others who have studied this fungus and other closely related species. These favorable conditions obtain in the mountain valleys of North Carolina in orchards which are so situated as to be shrouded in fogs until late in the morning for days in succession. In Florida, they obtain in groves which are surrounded by heavily-wooded swamps. Such situations provide excessively high relative humidities and at the same time, because of the surrounding forest, make impossible the draining away of the heavily moisture-laden air.

CONTROL

The nature of this group of diseases indicates that the use of sprays should prove effective in their control. In consequence, various investigators have attempted, with a considerable degree of success, to control them by the use of fungicides. Fawcett (10) recommends spraying coffee in Porto Rico to check *C. koleroga* with bordeaux mixture and respraying on the reappearance of the disease. Lime sulphur sprays and sulphur dusts were ineffective in his hands. Tunstall (21) and Shaw (16), however, found that lime sulphur sprays give satisfactory control, in India, of tea diseases caused by species of *Corticium*. Each recommends, though, that the spray be applied after pruning off and destroying the infected twigs.

By the use of bordeaux mixture in combatting *C. koleroga* on coffee, Rad (14) secured a heavy crop on sprayed blocks, whereas check blocks suffered severely. Coleman and his associates (3) report that bordeaux is fairly effective in combatting the same organism on coffee when the spraying is done before the break of the monsoon.

Winston (22) has reported that *C. koleroga* on grapefruit in Florida may be successfully checked by a single application, in May, of bordeaux-oil

(3-3-50 bordeaux mixture plus 1 per cent oil as emulsion). His experiments were conducted during the three succeeding seasons 1921, 1922, and 1923. The time of application varied each year because of rainfall and temperature conditions. Spraying was done as soon as the first indication of thread blight was apparent on the foliage. As a result the disease was abruptly checked and there was no further spread throughout the rainy season, although the organism persisted in the groves, probably by means of the sclerotia. These results have been confirmed by the writers during the season of 1926. A single application of bordeaux-oil made on the advent of the rainy season effectively checked the spread of this disease, and it did not reappear during the remainder of the summer.

Quaintance (15) has suggested that spraying with bordeaux mixture should control the disease on pears. Stevens and Hall (19) have noted that it does not occur in apple orchards which are systematically sprayed. This accords with the present observations, as it has been impossible to find affected trees in orchards in North Carolina in which the disease is known to have existed formerly. During recent years, however, such orchards have been sprayed several times during the course of each season. The fact that all specimens of thread blight on apples which have come to hand were taken in unsprayed orchards is regarded as additional indirect evidence of the effectiveness of fungicides.

SUMMARY

This investigation is concerned with a thread blight disease which occurs both on citrous and pomaceous plants and also on a wide variety of other hosts. Within the United States this disease was first recorded on *Citrus* in 1920, and on apple in 1907.

The disease appears on the leaves, twigs, and fruits. It is characterized, on apples, by the presence of brown rhizomorphs and sclerotia on fruits and twigs. Affected leaves are shed and dangle, being attached by rhizomorphs. The symptoms on *Citrus* are identical with those on apple except that sclerotia are less abundantly formed.

The thread blight fungus has been isolated from basidiospores from grapefruit, pear, pecan, and tung oil and found to be indistinguishable in cultures.

Pathogenicity has been proved by reciprocal inoculations with the organism from pear and grapefruit.

No detectable morphological differences have been noted between basidia and basidiospores from the several host species. Sclerotia from apple and grapefruit are of like structure.

The organism hibernates by means of sclerotia. The disease is locally disseminated by means of basidiospores.

The infection occurs by penetration of the stomates, and the mycelium is intercellular in grapefruit, pear, pecan, and pomegranate.

The cause of the thread blight disease is *Corticium koleroga*. This identification is based upon the similarity in morphological and cultural characters of the fungus from the several host species and of its ability to infect both grapefruit and pear. *Corticium stevensii* is held to be identical with *C. koleroga*.

Observations show that high humidities and high temperatures are necessary for its spread and development.

The thread blight disease on grapefruit in Florida has been satisfactorily controlled by a single application of 3–3–50 bordeaux mixture plus 1 per cent of oil as emulsion made on the advent of the rainy season.

OFFICE OF FRUIT DISEASE INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

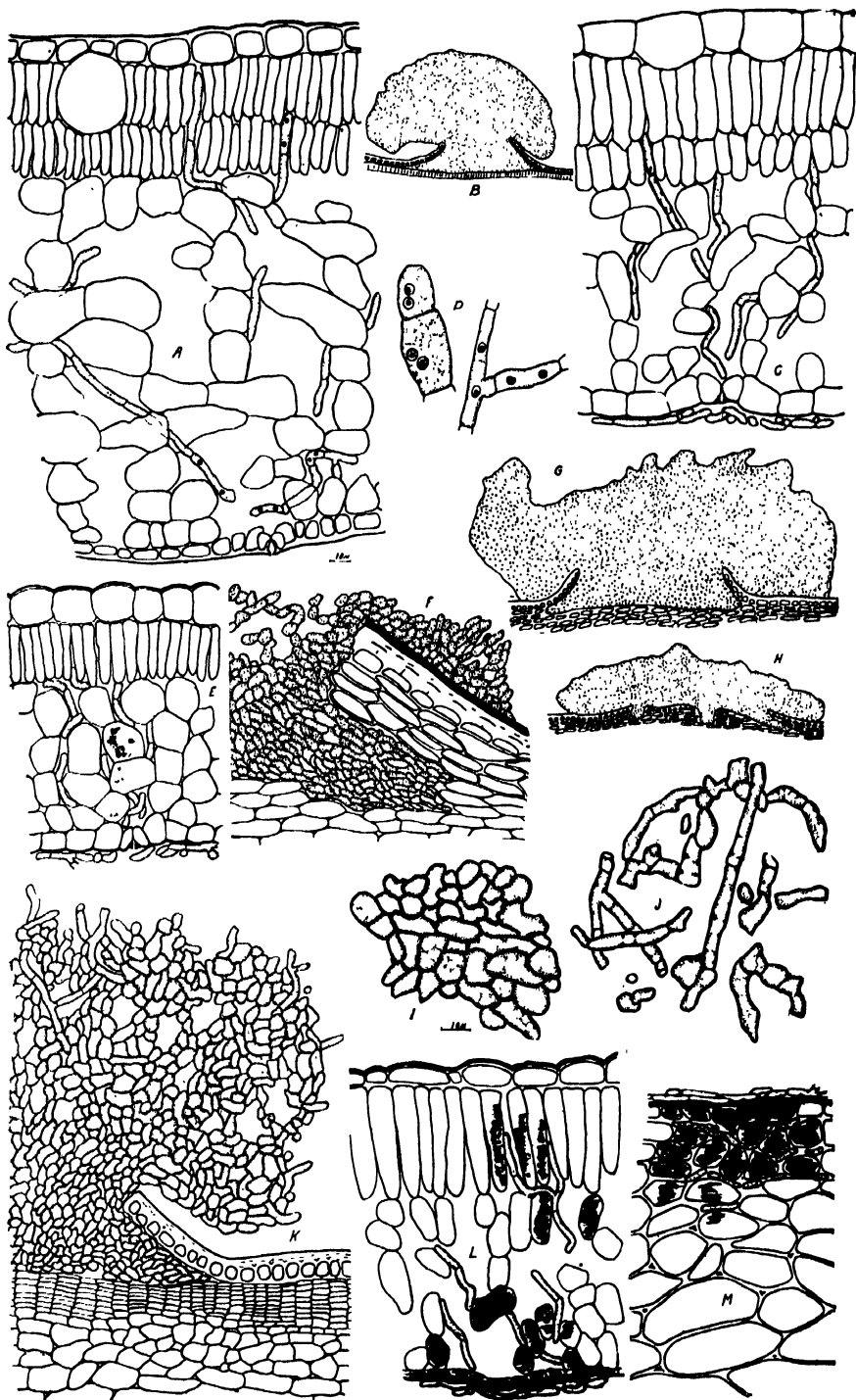
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EXPLANATION OF PLATE XXVI

- A, C, E, F, K, L, and M are drawn to scale below A. B, D, G, H, I, and J, to scale below I.
- A. Vertical section of grapefruit leaf attacked by thread blight. The mycelium occurs in the substomatal cavity and between the cells of the mesophyll and palisade parenchyma.
 - B. Diagram of sclerotium on grapefruit twig. The sclerotium is anchored in a cleft on the corky portion of the outer bark.
 - C. Vertical section of a diseased pear leaf. Manifestly infection occurs through the stomata, and the hyphae remain intercellular.
 - D. Binucleate cells from sclerotium and from intercellular hyphae from apple.
 - E. Section of diseased pomegranate leaf.
 - F. The basal portion, in vertical section, of a sclerotium on apple fruit, showing the relationship of the holdfast to the pulp cells.
 - G. Diagram of vertical section of sclerotium on apple fruit.
 - H. Diagram of large crustose sclerotium on apple twig, showing multiple holdfasts.
 - I. Compact fungous parenchyma from the basal portion of sclerotium.
 - J. Loose, hyphal tissue from medullary portion of sclerotium on apple.
 - K. Margin of the sclerotium shown in B, in detail.
 - L. Diseased pecan leaf in vertical section.
 - M. Rind of grapefruit, in section, from "scalded" area, showing intercellular hyphae and parasitized host cells.



COLOR MUTATIONS IN PUCCINIA GRAMINIS TRITICI (PERS.) ERIKSS. AND HENN.¹

MARGARET NEWTON AND THORVALDUR JOHNSON²

INTRODUCTION

Although mutations are known to occur commonly among fungi, no record seems to have been made of them in the Uredinales, or rust fungi. This may be due partly to the difficulty of recognizing mutations in rust. A rust might change its spore size, or even to some degree its pathogenicity, and the change still remain undetected. Should, however, a distinct color change take place in the spore, the mutation would much more likely be observed. In the course of determining the physiologic forms of wheat stem rust present in Canada, two interesting cases of this latter type came under observation. In one case, among the normal red pustules of the stem rust an orange pustule developed, and in the other case greyish-brown pustules appeared among the red.

ORIGIN OF MUTANTS

The orange mutant had its origin in a collection from Rosthern, Saskatchewan, which was gathered in July, 1926. For six generations this rust appeared to be a pure culture of *Puccinia graminis tritici*, physiologic form 9, with the characteristic red spores of all stem rust. In the seventh generation, or approximately four months after the culture had been started in the greenhouse, an orange pustule was observed among the red. This was isolated and obtained in pure culture, and for eight months it has continued to produce nothing but orange pustules (Plate XXVII). Although so changed in color, the mutant has shown no change in infection capabilities so far as the twelve standard differential host varieties are concerned. It still has the characteristic reactions of form 9, the form from which it came.

¹ Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

² The writers wish to acknowledge their indebtedness to Dr. A. T. Cameron, of the University of Manitoba, for suggestions in the chemical investigations; to Dr. C. H. Goulden for advice in determining the statistical constants; and to Mrs. D. L. Bailey for assistance in preparing the colored plate. The plate was made possible through a grant from the National Research Council of Canada.

The greyish-brown mutant³ was found in the first generation of uredinio-spores obtained from aecia on barberries at Winnipeg in July, 1926. A few scattered greyish-brown uredinia appeared among the numerous normal red uredinia. These were successively transferred to other wheat plants until a culture was obtained in which the pustules were all of one color. As in the former case, this rust has remained constant in color, producing only greyish-brown uredinia (Plate XXVII) for over eleven months; and, as before, has shown no demonstrable change in pathogenicity from the culture in which it arose; both the normal and the abnormal strains have a reaction identical with physiologic form 36.

At first it was thought that this abnormal color might be caused by some organism parasitizing the rust spores. Repeated attempts to isolate an organism from these spores failed. Attempts were made also to transmit this condition to normal rust. The procedure was based on the theory that the supposed organism was bacterial or virus in its nature, as no fungous parasite had been observed in connection with the spores. In either case it was thought that this condition could be transmitted to normal spores by means of a filtered extract of crushed greyish-brown spores. Consequently, the latter were ground as finely as possible in a mortar, and a suspension of them in distilled water was filtered through a Büchner filter to remove the spores. The filtrate was divided into two parts, to one of which were added normal spores. A number of susceptible wheat plants were sprayed with this spore suspension. Of 81 plants sprayed, 68 became infected, but all the uredinia were of the normal color type. The other half of the filtrate was used for spraying other plants as checks, on the supposition that some viable greyish-brown spores might have passed through the filter, but no infection took place on these plants.

DESCRIPTION OF MUTANTS

The colors of the uredinia of the normal and the aberrant rust forms were classified according to Ridgway's "Color Standards and Color Nomenclature" (7). The normal rust was classified as amber brown. The color varied, however, with the age of the pustules from Sanford's brown to burnt sienna. The orange rust was classified as orange, and the greyish-brown rust corresponded to Prout's brown. For convenience, however, the original terms red, orange, and greyish-brown will be used in this discussion.

³ During the month of June, 1927, while this paper was in preparation, inoculations from aeciospores of *Puccinia graminis* gave rise to greyish-brown uredinia similar to those described here. The physiologic form has not yet been determined. Until further information is available the term "mutant" is being used.

These differences in macroscopic appearance are accompanied by almost equally marked differences in microscopic appearance. The urediniospores of the orange rust are characterized by an entire lack of coloring-matter in the epispore, which appears hyaline after the germination of the spore. In normal rust the epispore is colored and the urediniospore retains a pale yellow color after germination. This lack of color in the spore wall of the orange spores probably accounts to a large extent for the difference in color between the two rusts. It is probable that the spore walls of the orange rust reflect more of the light than those of the red rust. The spore contents of the two rusts, on the other hand, appear similar in color. Hence, though the spores of the two rusts can be easily distinguished with the microscope where they are viewed by transmitted light, the difference is still more marked when seen by reflected light. The urediniospores of the greyish-brown rust also differ rather markedly from those of its red counterpart. The spore contents vary from grey to pale yellow in contrast with the bright orange of the normal spores. On germination they do not entirely lose their color, but remain a pale yellow like the normal spores. Great variations, however, occur in the size, shape, and color of the spores, a number of which are quite hyaline and non-viable.

FURTHER COMPARISON OF MUTANTS

Pathogenicity

As above mentioned, the infection capabilities of the abnormal rusts do not differ from those of the physiologic forms from which they mutated. It should be stated, however, that their relative pathogenicity has been tested only to the twelve differential hosts used in determining physiologic forms of *Puccinia graminis tritici* (11). No further comparison in infection type has been made between the normal rust and the mutants. Hence, as far as infection capability is concerned, the orange rust does not differ from the normal physiologic form 9, and similarly the greyish-brown rust answers to the infection type for form 36. The normal red rust produces spores in slightly greater abundance than do the two mutants, but this difference is not very appreciable and does not form a justifiable basis for distinguishing these from the red forms.

Spore Germination

A comparison was made of the viability of fresh urediniospores of the normal rust with that of the two mutants. The urediniospores were produced in the greenhouse on the thoroughly susceptible variety Little Club, and were tested for germination when the uredinia were about two weeks old.

TABLE 1.—Germination of normal, orange, and greyish-brown urediniospores of *Puccinia graminis tritici*

Normal				Orange			Greyish-brown		
Date of test, 1927	Total no. of spores	No. spores germinated	Percentage	Total no. of spores	No. spores germinated	Percentage	Total no. of spores	No. spores germinated	Percentage
Jan. 8	306	289	94.44	456	109	23.90	620	342	55.16
do 11	372	335	90.05	732	402	54.92	633	290	45.82
do 15	238	197	82.77	562	365	64.95	-----	-----	-----
do 19	500	332	66.40	1025	618	60.29	354	142	40.11
do 19	156	137	87.83	583	344	59.00	331	125	37.76
do 20	25	195	82.98	1039	738	71.03	846	367	43.38
do 25	-----	-----	-----	-----	-----	-----	505	299	59.21
do 26	368	322	87.50	934	550	58.89	725	434	59.86
do 28	369	332	89.97	604	461	76.33	641	373	58.19
do 31	423	378	89.36	792	702	88.64	459	293	63.84
Feb. 2	-----	-----	-----	433	259	59.82	432	294	68.06
do 4	333	306	91.89	717	401	55.93	410	219	53.41
Mean = 86.32				Mean = 61.24			Mean = 53.16		
				$M_N = 86.32$ $M_O = 61.24$ $M_{N-O} = 24.93$ $\sigma_{N-O} = 9.9182$ $t = 7.53$ Odds enormous			$M_N = 86.32$ $M_G = 53.16$ $M_{N-G} = 35.88$ $\sigma_{N-G} = 12.25$ $t = 9.27$ Odds enormous		

Some preliminary experiments were made to determine the best method for germination, tap water and distilled water being used in Syracuse watch glasses and in hanging-drops in Van Tieghem cells. Tap water was found preferable to distilled water, but little difference was observable between germination in the watch glasses and the hanging-drops, provided the latter were frequently aerated. The method finally adopted was to germinate spores in hanging-drops in the Van Tieghem cells for 48 hours, during which time the cells were lifted off the glass slide several times for aeration. The chief advantage in the use of the cells lies in the greater ease and accuracy with which the count can be made.

Table 1 represents the germination of the normal red, the orange, and the greyish-brown urediniospores. Each of the mutants, when compared with normal rust, shows a highly significant difference in the percentage of germination. The odds of significance were calculated from "Student's" tables of the distribution of t (12). Not only is the germination of the spores of both mutants considerably lower than that of the normal rust spores, but there is also a greater variation between consecutive tests than occurs in normal spores. The reason for this fluctuation is not clear, although it is possibly due to a more delicate response to adverse environmental conditions than exists in the normal spores. As the spores of both mutants are less viable than the normal spores, these fluctuations are not surprising.

Statistical Studies of Urediniospore Sizes

Methods of spore measurement.—The spores were projected through a microscope on to a paper screen. The magnification was standardized by means of a slide micrometer so that one small division (1/100 mm.) or 10 μ on the micrometer corresponded to one centimeter on the screen. Therefore one micron on the slide micrometer was represented by 1000 μ on the screen. By means of calipers and a steel rule the measurements of the spores were obtained in microns at a magnification of 1000.

In preliminary experiments to determine what represented accurate random sampling of the urediniospores for length and width, it was found that considerable variation occurred among samples of 100 spores when taken from single pustules of the same host. Increasing the size of these samples did not overcome the difficulty, but when four or five of the small samples from the different pustules were combined, uniformity was obtained in curve type as well as in the magnitude of the calculated statistical constants.

According to Levine (3), urediniospores of a single physiologic form from thoroughly susceptible hosts do not differ in type even if the hosts are

different varieties, although spores of the same form from a resistant variety are markedly different in type. Consequently, in this study, the spores were collected only from susceptible hosts, the variety Little Club being used throughout. All the plants were grown under identical conditions, and uredinia of the same age were used as a source of spore material. Populations of 500 spores were thought sufficiently large. Each of these populations was composed of five random samples of 100 spores each. The spores were mounted in glycerine and measured immediately after mounting.

Spore sizes of form 9, red and orange.—Two series of measurements were made of the red and orange rusts. The first series of measurements, represented by figure 1, was made in January when light conditions were

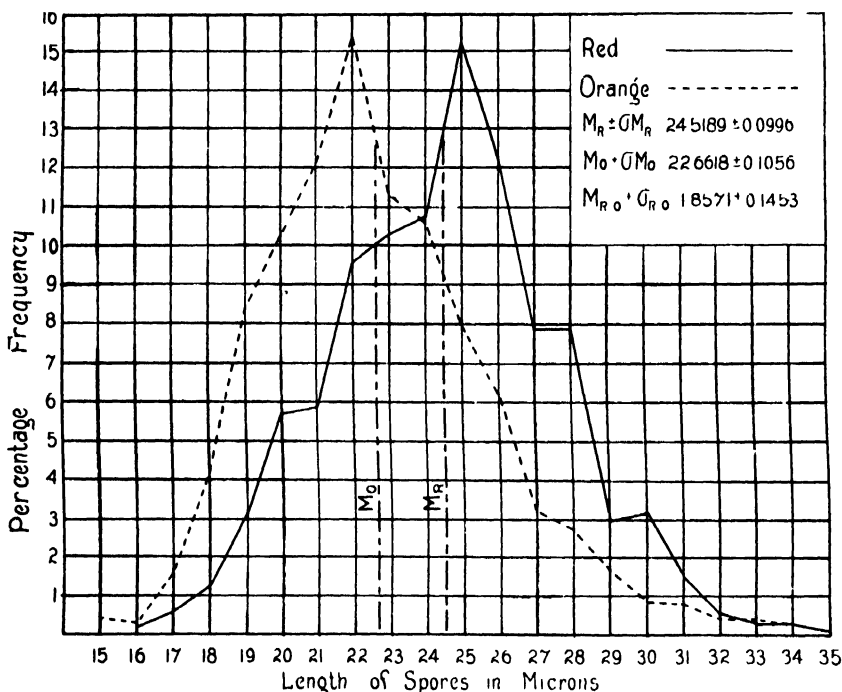


FIG. 1. Percentage frequency distributions for length of 979 spores of form 9 red (normal), and 887 spores of form 9 orange (mutant).

rather poor for rust development. The second series of measurements, as shown in figure 2, was made more than two months later, in April, when light conditions were almost ideal for the development of host and fungus. Both length and width were measured, but as the variations in width did not appear significant, the graphs are concerned only with the length.

The frequency distributions for length and width of spores are given in table 2.

Figure 1 shows plainly a significant difference between the lengths of the red and orange spores. The measurements of the widths of the spores also seem to show a significant difference. The mean width of the red spores in microns is 14.2520 ± 0.0669 , that of the orange spores 14.7099 ± 0.0604 . The difference between the means is 0.4579 ± 0.0280 .

TABLE 2.—*Frequency distributions for length and width of urediniospores of form 9 red and orange. First series of measurements*

Length of spores			Width of spores		
Microns	Form 9 red	Form 9 orange	Microns	Form 9 red	Form 9 orange
15	...	4	8	1	..
16	2	3	9	5	..
17	6	14	10	30	5
18	13	38	11	69	27
19	31	75	12	119	64
20	56	91	13	140	131
21	58	108	14	142	167
22	94	137	15	175	205
23	101	102	16	154	155
24	106	94	17	101	74
25	149	71	18	43	41
26	120	55	19	4	14
27	77	29	20	1	3
28	77	25			
29	29	15			
30	32	8			
31	15	7			
32	6	4			
33	3	4			
34	3	3			
35	1			
Totals . . .	979	887		984	886

Figure 2, as stated above, represents the second series of measurements of the spores of the red and orange rusts made two months later under more favorable conditions for spore development. This shows an even more significant difference between the lengths of the spores of the normal rust and of the mutant than the first series. It will be noticed that the mean lengths, in the case of both rusts, are somewhat greater than in the earlier measurements. This may be attributed probably to more vigorous spore development under conditions of longer daylight and more congenial temperature. The mean width in microns is 14.6934 ± 0.0868 for the red

spores, and 14.3300 ± 0.0738 for the orange. The difference between the means is 0.3634 ± 0.1139 . This difference should probably not be considered significant, especially as it is directly opposite to that secured in the first measurement of the spore width of these two rusts. In general, although the measurements for spore width are reported, the writers prefer to draw conclusions only from the more obviously significant differences in spore length.

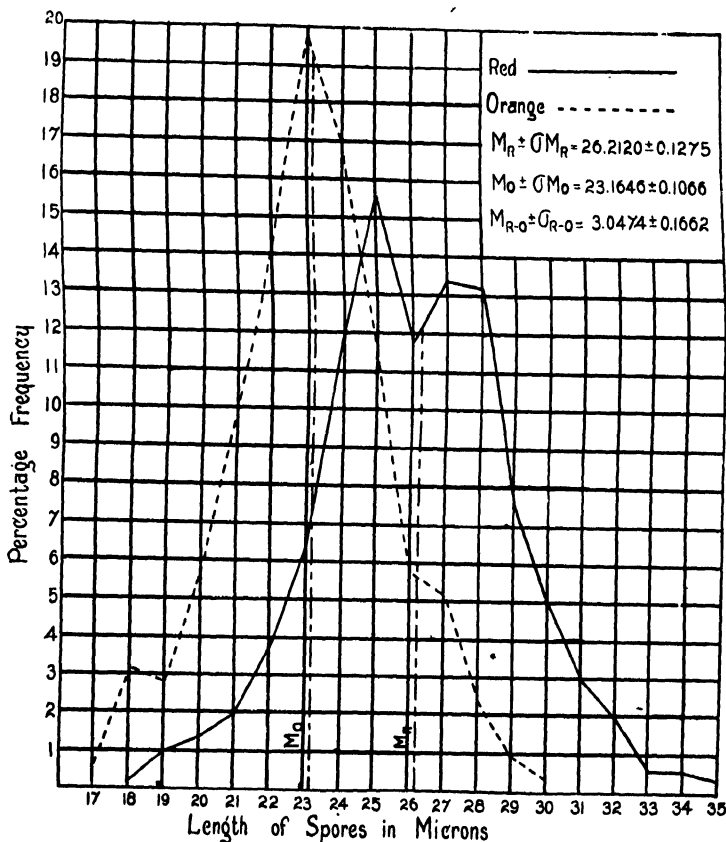


FIG. 2. Percentage frequency distributions for length of 500 spores of form 9 red (normal) and 498 spores of form 9 orange (mutant). Second series of measurements.

The frequency distributions for spore length and width as obtained in the second set of measurements are given in table 3.

Spore sizes of form 36, red and greyish-brown.—Figure 3 represents measurements of 500 spores of the normal form 36 and an equal number of spores of its greyish-brown mutant. The figure shows a significant difference in spore length. Both measurements are represented by a normal type

of curve, although that of the mutant shows a greater range than that of the normal rust form. This is due to the greater variation in length and shape of the spores of the mutant, and these variations are quite noticeable on examination with the microscope. The measurements of the widths of the spores reveal a difference which is, however, of doubtful significance. The mean width of the red spores in microns is 15.1142 ± 0.0843 , that of the greyish-brown 14.7120 ± 0.1086 . The difference between the means is 0.4022 ± 0.1375 .

TABLE 3.—*Frequency distribution for length and width of urediniospores of form 9 red and orange. Second series of measurements*

Length of spores			Width of spores		
Microns	Form 9 red	Form 9 orange	Microns	Form 9 red	Form 9 orange
17	3	9	...	1
18	1	16	10	4	1
19	5	14	11	17	18
20	7	29	12	47	53
21	10	50	13	79	85
22	19	71	14	82	99
23	32	99	15	105	126
24	60	85	16	77	72
25	78	57	17	53	31
26	59	29	18	21	14
27	67	26	19	11
28	66	12	20	3	
29	38	5			
30	25	2			
31	15				
32	10				
33	3				
34	3				
35	2				
Totals	500	498		499	500

The frequency distributions for length and width of the spores of the normal red and the abnormal greyish-brown rust are given in table 4.

Nuclear condition of mutants.—Dodge (1), working with *Caeoma nitens*, the orange rust on *Rubus*, was able to show that in two of the short-cycled rusts which he investigated a definite color of the aecium was often associated with certain nuclear phenomena and with certain spore sizes. Usually in the reddish-orange sori there occurred chiefly binucleated spores; while in the yellowish-orange aecia, chiefly uninucleated spores. Also, when

the aecia were yellowish-orange as contrasted with those of reddish-orange color, the spores were found to be smaller and less uniform in size and shape.

Although Dodge was dealing with a different rust and a different stage in the cycle of the rust from that studied by the writers, it was thought advisable, owing to similar differences in spore size and color, to make a cytological study of the urediniospores of the normal rust and of the two mutants.

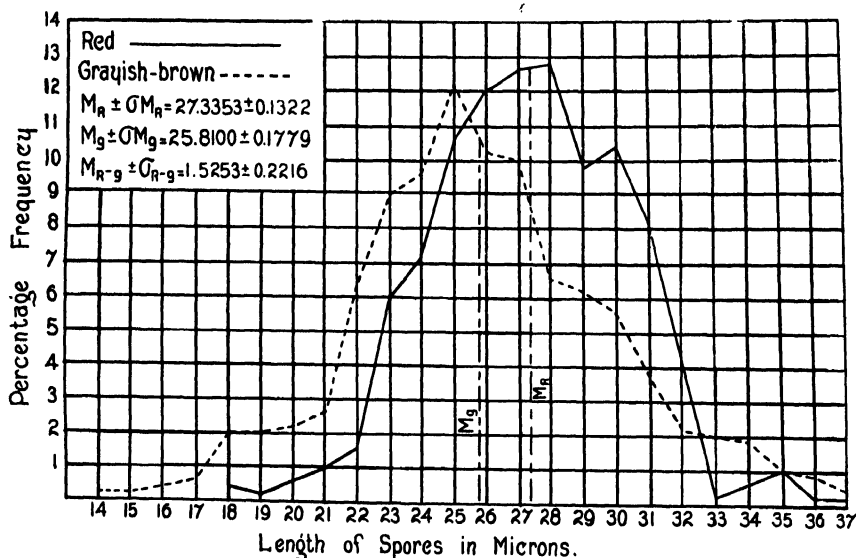


FIG. 3. Percentage frequency distributions for length of 500 spores of form 36 red (normal), and 500 spores of form 36 greyish-brown (mutant).

Little Club, a wheat susceptible both to forms 9 and 36 as well as to both mutants, was used for all infections. When the pustules were just breaking through the epidermis of the wheat plants, the leaves were embedded in paraffin in the usual manner, and sections cut from 5 to 10 μ thick. Iron-alum haematoxylin was the chief stain used.

A study of a large number of slides revealed nothing unusual in the nuclear condition of the urediniospores of either the orange or the greyish-brown mutant. In the abnormal as well as in the normal rust, all the younger spores were distinctly binucleated (Fig. 4).

PRELIMINARY CHEMICAL INVESTIGATION OF THE COLORED COMPOUNDS IN THE NORMAL RUST AND IN THE MUTANTS

The appearance of the spores of these rusts suggests the presence of carotin or xanthophyll or both, lycopin or rhodoxanthin being also possibilities. The following tests have therefore been carried out:

TABLE 4.—*Frequency distributions for length and width of urediniospores of form 36 red and greyish-brown*

Length of spores			Width of spores		
Microns	Form 36 red	Form 36 greyish-brown	Microns	Form 36 red	Form 36 greyish-brown
14		1	8		1
15		1	9		2
16		2	10	1	18
17		3	11	13	27
18	2	10	12	37	50
19	1	10	13	58	64
20	3	16	14	68	65
21	5	18	15	96	87
22	8	32	16	101	64
23	30	45	17	76	60
24	36	48	18	41	30
25	53	61	19	7	20
26	60	51	20	1	10
27	63	50	21		2
28	64	33			
29	49	31			
30	54	28			
31	41	19			
32	21	11			
33	1	10			
34	3	9			
35	5	5			
36	1	4			
37	1	2			
Totals	500	500		499	500

(a) Extraction with carbon bisulphide gave orange-colored solutions with the normal rust and the orange mutant, but no colored material was extracted from the greyish-brown rust. Using equal weights of the powdered rust material of the first two and equal volumes of carbon bisulphide, solutions of almost the same intensity were obtained, and these examined in a Hilger constant deviation spectrometer showed a band in the correct position for carotin, the red end being in two series of experiments, respectively 537 and 537.5 μ for the normal rust, and 536 and 536 μ for the orange. The other end could not be determined accurately. No bands were observed in the solution from the greyish-brown rust. Willstätter and Stoll (13) state that the corresponding carotin band has the limits 524–510 μ in carbon bisulphide. This result suggests that carotin may be pres-

ent to an equal extent in both the normal rust and the orange mutant, but is certainly absent from the greyish-brown mutant. [The spectrometer had too wide a dispersion to distinguish the band in the blue—*Vid.* Palmer (6), p. 221.]



FIG. 4.—Section through a uredinium of the greyish-brown mutant $\times 700$. (Photomicrograph by Dr. A. Savage.)

(b) Eighty per cent methyl alcohol extracted no color from any of the three. According to Jörgensen and Stiles (2), xanthophyll, if present, should be extracted. Xanthophyll therefore appears to be absent from these rusts, and this result shows further that the band seen in the spectrometer was not due to xanthophyll, strengthening the evidence in favor of carotin.

(c) Alcohol or acetone should give a rose pink extract if rhodoxanthin is present. No such color has been observed in alcoholic or acetone solutions from any of these rusts.

(d) The position of the band observed definitely excludes lycopin from the normal and the orange rust, and the absence of extractable material with carbon bisulphide also excludes it from the greyish-brown rust.

(e) Addition of concentrated sulphuric acid to powdered normal rust gave a slight blue color, while the orange mutant gave a much more intense blue, and the greyish-brown no trace of blue color. According to Palmer (6) this test is given by both carotin and xanthophyll. The difference of

intensity obtained under comparable conditions with the first two rusts, while confirming the probable presence of carotin, suggests that some additional reacting substance may be present in the orange mutant.

(f) Monteverde and Lubimenko (4) state as a specific test for xanthophyll that a green color is produced on the addition of formic acid, but, as Palmer (6) points out, this test has been mentioned only by these investigators. The writers found that the orange rust gave a very slight positive reaction, the normal rust a somewhat stronger reaction, and the greyish-brown a negative reaction, while dandelion heads employed as source of xanthophyll (6, 10) gave a much more intense color. In the absence of further evidence as to the specificity of this reaction, the writers doubt if definite conclusions can be drawn from their results with it.

(g) The powdered greyish-brown mutant has been treated with other organic fat solvents—acetone, ether, chloroform, alcohol, etc., and with boiling water, and boiling dilute acetic acid. The red coloring material was not extracted by any of these reagents. Similar treatments of the powdered rust spores after the orange coloring matter had been removed by carbon bisulphide failed to extract the red. Both carbon bisulphide and acetone removed all the color from the orange rust, leaving it a chalky white. It seems, therefore, that the parent normal rust has at least two distinct color substances, orange and red, while neither of the mutants possesses both. The normal rust and the orange mutant have orange in common, apparently located in the cytoplasm of the spore. The normal rust and the greyish-brown mutant have red in common, apparently located in the spore wall and insoluble in any of the reagents used.

Conclusions. The above evidence suggests strongly that the normal rust and the orange mutant contain carotin, this being responsible for the orange color. On the other hand, since equal weights of these two rusts extracted by equal amounts of carbon bisulphide gave colored solutions of equal intensity with a corresponding absorption band, it would appear that carotin can not be responsible for the prevailing color of the normal rust. As it was not possible to extract the red color from either the normal rust or the greyish-brown mutant by the treatments employed, it seems possible that there is present in both of these some unknown red compound which is markedly resistant to solvents, which is neither carotin, xanthophyll, lycopin, nor rhodoxanthin, and indeed is not a carotinoid. The authors are continuing work to endeavor to ascertain something of the nature of this coloring matter.

SUMMARY

1. Two color mutations, one of which is bright orange and the other greyish-brown, have been observed in the uredinial stage of *Puccinia grami-*

nis tritici. The aecial and telial stages of the mutants have not yet been studied.

2. The orange mutant appeared as a single pustule in a culture which for six generations of uredinia had produced spores of normal color; the greyish-brown mutant appeared in the first uredinial generation of a culture derived from aecia on *Berberis vulgaris*.

3. Although so changed in color, the mutants have shown no change in infection capabilities so far as the twelve standard differential host varieties are concerned. The orange mutant still has the characteristic reaction of physiologic form 9, from which it arose; and, similarly, the greyish-brown mutant remains identical pathogenically with form 36.

4. Both mutants differ markedly from the normal rust in the viability of their urediniospores. The average germination of the normal urediniospores was 86.32 per cent, of the orange mutant 61.24 per cent, and of the greyish-brown 53.16 per cent.

5. There is a significant difference in size between the urediniospores of the normal rust and those of the mutants, the former being markedly longer, but of approximately the same width.

6. The urediniospores of both mutants are binucleate.

7. Microscopic examination shows that the spore walls of the orange rust are colorless, while in the greyish-brown and in the normal rust they are colored. On the other hand, the cytoplasm of the orange and the normal spores is colored, while that of the greyish-brown spores is practically colorless.

A chemical investigation adduced strong evidence that carotin was present in the normal and in the orange spores, but not in the greyish-brown spores. The reddish-brown color in the normal and the greyish-brown spores appears to be due to some unidentified compound which is not a carotinoid, although of course the identity of the two reddish-brown colors can as yet only be assumed.

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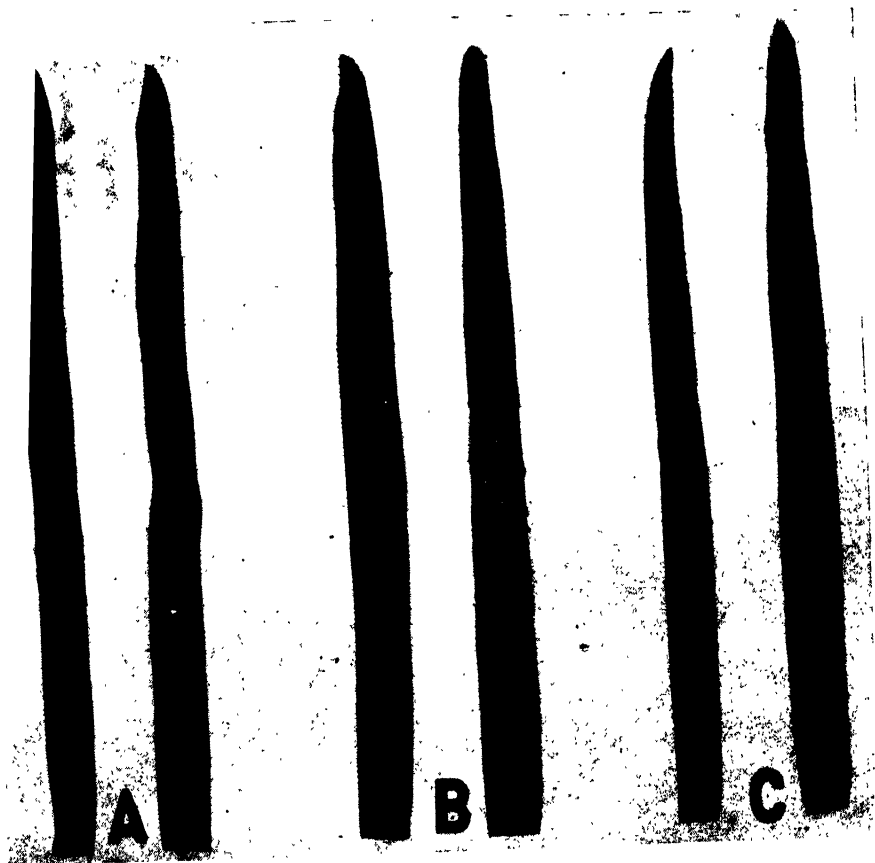
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EXPLANATION OF COLORED PLATE XXVII

Leaves of Little Club wheat inoculated with *Puccinia graminis tritici*:

- A. Normal rust.
- B. Orange mutant.
- C. Greyish-brown mutant.



Most of the striking differences in color between A (normal rust) and C (grayish-brown mutant) have been lost in reproduction. See "Description of Mutants" in text for exact colors.

TOBACCO MOSAIC ON POTATOES

F. M. BLODGETT

In several recently published papers there are some seeming discrepancies in the results secured by inoculating potatoes with the tobacco mosaic virus. Johnson (6) states that "Tobacco mosaic produces brown or black necrotic lesions on the stems and petioles of potatoes apparently at the points of inoculations. Tobacco mosaic infection was not found to be systemic in potato, however, and it cannot therefore be said to be a typical host of tobacco mosaic." On the other hand, Fernow (5) reports symptoms that closely parallel those of streak of potatoes as described by Schultz and Folsom. "The petioles and the young stems become affected with a severe necrotic streaking, and affected shoots generally die soon after. . . . The second generation plants are usually much dwarfed and the leaves may show from one to five yellowish spots which become brown. . . . The petioles and stems are often affected with a more severe necrotic streaking than in the first generation plants, and the older leaves are dropped, leaving only a small crown." In this case the virus is obviously systemic, as the entire plant is affected, and the virus is carried over by the tubers to the second generation. Quanjer (8) concluded that tobacco mosaic is identical with or at least closely related to potato mosaic. This conclusion seems to have been reached as the result of a transfer of mosaic from tomato to one variety of potato by grafting, but it is not clear in one paper (8, p. 42) whether the mosaic transferred was tobacco mosaic. In a paper by Quanjer (9, p. 26) where reference is made to the same or another experiment of a similar kind, potato mosaic seems to have been the one transferred, although it is perhaps equally likely that the disease transferred was stipple-streak. This suggests itself as Quanjer reports obtaining this transfer only from the variety Zeeuwsche Blauwe and a failure from the varieties Eigenheimer and Bravo. Of the variety Zeeuwsche Blauwe, Atanasoff (2) says: "the variety as far as studied by the writer and perhaps in its entirety is infected with stipple-streak and always carries its virus, although under natural conditions it never shows the slightest symptoms of disease." Clinton (3) reports one positive case of infection with tobacco mosaic on a potato seedling. A number of unsuccessful attempts to inoculate potatoes with tobacco mosaic virus have been reported in literature by Allard (1), Dickson (4), Clinton (3), and Schultz and Folsom (10).

Apparently rather puzzling is the fact that Fernow (5) and Johnson (6) report quite different results from inoculating potatoes with tobacco mosaic virus, as there seems to be good reason to believe the work was well done in both cases and sufficient numbers used to give reliable results. Some experiments performed by the writer in 1920, and not previously reported, have a bearing on this discrepancy. In January, 1920, there were growing in the greenhouse eight tuber units of potatoes of the Green Mountain type selected from a relatively healthy commercial field. These were numbered 50 to 57, and the pieces from each potato labelled A, B, C, D. On January 21, tomato cions affected with tobacco mosaic were grafted on plants 52C, 53C, and 56C. The inoculated plants thus belonged to different tuber units. The plants lettered A and the remainder of the plants lettered C were treated in various other ways which need not be detailed here. The B and D plants of each tuber unit were left as untreated checks. All of these plants were in small pots, made little further growth, and no symptoms of infection were noticed in any of them. All plants were dug in April and planted again on July 13 of the same year. The tuber progeny of each plant of the previous generation was kept separate. Altogether there were in the series 82 plants which were tuber progeny of 30 plants in the previous series. Six of these were from the grafted plants, 52C, 53C, and 56C. Eleven plants were from the uninoculated B and D plants in the same tuber units, 52, 53, and 56, and thus served as checks on the original condition of these units with respect to freedom from mosaic diseases. All of these plants, except the 6 plants from 52C, 53C, and 56C, were large and healthy and grew vigorously. The six plants from the graft-inoculated plants were strikingly different. They were minute dwarfs, never exceeding about 5 inches in height. The leaves started to unfold, but instead of completing the process, they turned yellow and dropped soon after. The resultant plant was small, bare stemmed, with streaking on stems and petioles, and with a small tuft of partly unfolded leaves at the top. Thus these results seem to agree with those reported by Fernow, in that the inoculation of potatoes with tobacco mosaic virus produced a systemic invasion, the virus being carried over through the tubers, and the symptoms in the tuber progeny resembling those of streak of potatoes. It should be noted that these plants were grown in the greenhouse in the summer time so that rather high temperatures prevailed and thus, as will be seen later, symptoms typical of high temperature conditions appeared.

In the spring of 1926, after the papers by Johnson and Fernow had been published, and the question of the seeming discrepancy of results was raised, further inoculation experiments were undertaken with the hope of clearing up the matter. To secure tobacco mosaic, a mosaic tobacco extract

bottled in August, 1920, was used to inoculate tobacco plants. It was believed that this extract, because of its age and the known longevity of the tobacco mosaic virus as compared with certain other viruses, would be as certain a source of the unmixed virus of tobacco mosaic as could be readily obtained. The inoculated tobacco plants developed typical tobacco mosaic.

The method of inoculation from mosaic tobacco to potatoes was essentially the same as that described by Johnson (6). Mosaic tobacco leaves were crushed in a small amount of water with a steam sterilized mortar and pestle. The inoculations were made with a flame sterilized needle on which a small wad of absorbent cotton was wound near the tip. Sterile forceps were used to hold the stems or leaves of the plant to be inoculated where this was required. This method has been used rather extensively in other experiments. It seems to be an effective way of making inoculations with a number of viruses and introduces very little danger of contamination.

In this manner five Bliss Triumph potato plants were inoculated in the greenhouse with the tobacco mosaic virus from tobacco on April 2, 1926. The plants were 6 to 8 inches high, grown for indexing purposes under conditions favorable for the development of mosaic symptoms, and had been selected as healthy. They were transplanted to large pots before inoculation so conditions would be favorable for continued growth. At the same time inoculations were made with five other viruses on other Bliss Triumph potatoes, and five check plants were punctured with the same needle sterilized in the usual way. Numerous other plants of the same lot remained uninoculated. Inoculations were made by puncturing the plants rather freely, ten punctures in each of several lower nodes and in each of three leaflets. Within a week, necrotic lesions were noted around the points where inoculations were made with tobacco mosaic; and within a month some of the lower leaves had turned yellow and fallen. There did not, however, seem to be any indication of systemic infection at any time. The lower leaves that turned yellow were those which had been punctured in the base of the petiole or in which the punctures in the stem were so close to the petioles that the necrotic lesions about the punctures might easily have affected the leaves. These inoculated potato plants continued to grow and showed no further symptoms of disease. Their progeny are now being grown and appear to be healthy. None of the check plants and none of the plants inoculated with other viruses showed these local necrotic lesions at the points of inoculation.

The results in this test are in accord with those reported by Johnson (6). This suggested at once that the variety of potatoes used is an important factor in the results, for Johnson apparently used only Bliss Triumph potatoes for his experiments with tobacco mosaic on potatoes. The results also suggest that in this variety the rapid killing of the tissues about the

point of inoculation with tobacco mosaic virus may protect the plant in some way from becoming systemically invaded.

On April 7, when it had already become evident that different results were being secured on Bliss Triumphs than had previously been obtained on Green Mountains, inoculation experiments were extended to include No. 9 potatoes (a variety belonging to the Rural group). Eight plants of this variety inoculated, as previously described, with tobacco mosaic virus did not develop necrotic lesions at the points of inoculation, although some of the lower leaves turned yellow and dropped. Necrotic lesions developed at the tips of a few leaflets, and by May 4 such lesions had developed in the pith and nodes of the upper parts of the stem. On the new leaves of three of the plants there was a striking mosaic mottling. Later, mottling appeared on all plants. This mottling differed from types seen previously by the writer on potatoes, and resembled more nearly the usual mottling of mosaic on tobacco. The spots were rather large, sometimes involving half a leaf. The degree of malformation corresponded with the size of the spots. The color contrast between the light green and dark green areas was marked. These plants were in a high temperature greenhouse until symptoms began to appear. Some of them were then moved to a cooler house, but even there low temperatures could not be maintained at that time of year. Eight punctured check plants remained healthy and normal.

Some of the inoculated No. 9 potato plants were cut back and transferred to the field. The new leaves on side branches which had grown after the time of inoculation were later used as a source of inoculum for tobacco in the field. The inoculations made on tobacco in the field (July 22) were as follows: Five tobacco plants inoculated with tobacco mosaic virus from tobacco all became typically affected with tobacco mosaic (Aug. 3); ten tobacco plants inoculated from No. 9 potato affected with tobacco mosaic all became typically affected with tobacco mosaic (Aug. 3), and symptoms were not distinguishable from those on plants inoculated from tobacco. Of ten check plants alternating with the ten plants just mentioned, one became affected with tobacco mosaic, and nine remained healthy. Ten tobacco plants were also inoculated from healthy No. 9 potatoes and ten more from healthy Green Mountains, with numerous additional uninoculated checks. Under field conditions, no symptoms could be detected with certainty on these plants. Certainly nothing developed in any way resembling the tobacco mosaic referred to above. As a rule, such inoculations made from healthy potatoes to tobacco under greenhouse conditions have given a mosaic on tobacco with symptoms of Johnson's "mottle" type, which is readily distinguishable from tobacco mosaic.

At the time the inoculations of tobacco were made in the field, a number of potatoes were also inoculated. Of ten tuber units of No. 9 potatoes, one

stalk in each of two plants in each unit was inoculated with tobacco mosaic virus from tobacco. One half of the potato plants was inoculated with material from the same tobacco plant which furnished inoculum for the Bliss Triumph earlier in the season. The other half was inoculated with material from young tobacco plants on which symptoms were just beginning to appear after inoculation with tobacco mosaic from the bottle of old extract.

Parallel inoculations were made at the same time on an equal number of Green Mountain potatoes. These, however, were not tuber units but the ordinary run of seed from a relatively disease-free stock. Pairs of inoculated plants alternated in the row with pairs of check plants.

The inoculated Green Mountain potatoes developed symptoms a few days earlier than the Rurals and were showing rather marked streaking and spotting of stems and leaves during the week of August 13 to 20. These stalks were so severely injured that they made little or no further growth and died early in most cases, while the check plants and the uninoculated stalks in the same hills continued to grow vigorously.

All of the No. 9 potatoes inoculated with tobacco mosaic virus showed symptoms similar to those previously obtained in the greenhouse. In comparison with the checks they were considerably dwarfed. They continued, however, to make some new growth, and on this the leaves were mottled. The checks remained healthy with the exception of one unit which seemed to be affected with leaf roll, probably tuber transmitted.

Tuber progeny of some of the No. 9 and Green Mountain potatoes inoculated with tobacco mosaic virus in the field were grown in the greenhouse during the following winter. Under greenhouse conditions it soon appeared that temperature has a very important influence on the type of symptoms developed. Both No. 9 and Green Mountain potatoes grown at 15° to 18° C. appeared quite normal in the early stages of growth. Later a rolling of the leaves gradually developed, with a slight crinkling of the leaf surface. No mottling or necrosis was observed at this temperature. At a temperature ranging from 26° to 30° C., on the other hand, the two varieties behaved differently. The No. 9 potatoes, in addition to a general dwarfing, developed a very striking mottling of the leaves. The Green Mountain potatoes developed a necrotic streaking of the stems and spotting of the leaves as previously described. This occurred both in cases when potatoes were planted and kept in a warm house continuously and when they were brought into a warm house after having been started in a cool house. In the latter case the No. 9 potatoes developed mottling only on the leaves that grew after the potatoes were removed to the warm house. At the same time it was found that when inoculations were made with tobacco mosaic virus on potatoes of several varieties in the cool house, symp-

toms did not develop until they were removed to the high temperature house. Thus, while no systematic study has been made of the effect of temperature on the tobacco mosaic disease on potatoes, enough has been done to indicate clearly that it is a high temperature disease—which seems to distinguish it from the other mosaics of potatoes, at least from those whose temperature relations have been described. Yellow dwarf, which is also a high temperature disease, has quite different symptoms.

Inoculations were made on tobacco in the greenhouse from the progeny of some of the potatoes that had been inoculated with tobacco mosaic virus. The following are typical of the results secured. Five young tobacco plants inoculated with tobacco mosaic virus from tobacco were affected with typical tobacco mosaic. Five tobacco plants inoculated from No. 9 potatoes, progeny of potatoes inoculated with tobacco mosaic virus in the field, were affected with a combination mosaic as described by Johnson, characterized by early appearance of symptoms and considerable necrotic leaf spotting in addition to mottling. Five tobacco plants inoculated from No. 9 potatoes, progeny of potatoes inoculated with tobacco mosaic virus in the greenhouse, were affected with the combination mosaic. Five tobacco plants inoculated from Bliss Triumph, progeny of potatoes inoculated with tobacco mosaic virus in the greenhouse, were affected with a faint mottling of the larger leaves (described by Johnson as "mottle"), like that obtained on tobacco most commonly when it is inoculated from apparently healthy commercial potatoes. Ten uninoculated tobacco plants remained healthy. From these results it appears that the tobacco mosaic virus is transmitted by the tubers of No. 9 potatoes but not through those of Bliss Triumph.

In addition to results above reported, some preliminary observations were made on the effect of tobacco mosaic on other varieties of potato. Ten plants of each variety were inoculated, and ten kept as checks. The progeny of these potatoes have not yet been grown, and the results were not so clear cut as might be desired. Five plants of each variety were inoculated in the cool house (65° F.), but after about a month, as no symptoms were evident, they were removed to the warm house. In addition, another five plants of each variety were inoculated in the warm house. The inoculated plants of the Spaulding Rose variety were dwarfed, and necrotic lesions developed on the stems at the points of inoculation. The tops died irregularly, but the stems in many cases sent out new sprouts near the surface of the soil which were nearly normal in appearance, although there was a faint mottling and crinkling in some cases. The inoculated Early Ohio potato plants died quickly, and there was necrotic streaking of stems and spotting of leaves. In two cases new shoots developed which for a short time showed striking mottling, comparable with that developed on No. 9 potatoes, but these shortly

developed necrotic streaking and spotting also. Cobblers inoculated with tobacco mosaic virus all died at somewhat irregular intervals. Death followed the development of extensive necrotic areas in the stems. No mottling or spotting of the leaves was noted. Most of the Early Bovee potatoes inoculated with tobacco mosaic virus were alive after five months although somewhat dwarfed compared with the check plants. They also gradually developed a rolling of the leaves. The new leaves, of a few new shoots, were mottled and within a short time the tissue in the light areas died.

So far this tobacco mosaic disease has not been definitely connected with any disease occurring in commercial fields of potatoes. The symptoms on Green Mountains, however, so closely resemble those of the streak disease as to suggest at once that some part of this disease may be tobacco mosaic. The fact that the symptoms of tobacco mosaic vary so greatly on different varieties of potatoes and vary also under different temperature conditions makes possible a wide variety of symptoms under varying field conditions. Such a symptom complex has recently been described for the streak disease of potatoes in Europe by Atanasoff (2) under the name stipple-streak. However, as most of the potato varieties used are different from ours and no reference was made to the temperature conditions under which the work was done, it is not possible to say whether this is tobacco mosaic until further comparisons can be made. The marked effect of temperature, the successful infection of tobacco, the diverse symptoms on the three principal varieties of potato seem to offer good means of identifying this disease.

SUMMARY

The author's results agree with those obtained by Johnson and with those obtained by Fernow. The seeming difference arose from the fact that Johnson had used Bliss Triumph potatoes for inoculations with tobacco mosaic, while Fernow had used Green Mountains.

Tobacco mosaic produced different symptoms on different varieties of potato.

On Bliss Triumphs, local necrotic lesions were produced at the points of inoculation, with no systemic infection.

On Green Mountains the symptoms were much like those described for streak, consisting of streaks on the stems and veins and necrotic spotting of the leaves. This is usually accompanied in the second generation by extreme dwarfing and dropping of leaves. This raises the question as to whether some part of the disease diagnosed as streak in potatoes may not be tobacco mosaic.

On No. 9 potatoes, in addition to a limited amount of necrotic streaking and spotting, a mottling developed which resembled that of tobacco mosaic

on tobacco rather than the usual types observed on potatoes. They were also slightly stunted.

Preliminary experiments on other varieties of potato indicate that the tobacco mosaic virus causes a wide range of symptoms.

Marked symptoms were obtained only at relatively high temperatures, about 26° C. and above. No symptoms could be detected in young plants at temperatures from 15° to 18° C.

Tobacco mosaic virus is readily transmitted from potatoes back to tobacco, although in doing this one obtains not the tobacco mosaic disease alone but a combination disease consisting of tobacco mosaic and the virus disease commonly carried by apparently healthy commercial potatoes.

The needle puncture method, when rather numerous punctures are made, is effective for inoculating the tobacco mosaic virus into potatoes.

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"TARGET CANKER" OF APPLES AND PEARS

JOHN W. ROBERTS

The canker of apples and pears herein described under the name "target canker" is not a new one, but because it seems not to be generally recognized and because of its unusual prevalence in recent years it appears worthy of some attention.

The disease first came to the writer's attention in 1922 when he noticed two apple trees at Arlington Farm, Virginia, the trunk and older branches of which were almost completely covered with bark cankers. On both trees these cankers consisted of circular dead areas of bark in which there were cracks arranged in concentric circles. The concentricity of these circles was so nearly perfect that it suggested the name "target canker." In August of that year, on the current year's growth of water-sprouts, young cankers were noted which, starting as dark pimples, soon showed a characteristic arrangement of concentric circular ridges which later cracked open (Fig. 1).

The cankers are confined to the cortical layers and become more superficial with age, since they are cut out by the formation of cork underneath. Even cankers of the current year's growth collected in July have been found to be isolated by a layer of cork. On older twigs the cankers appear to start as raised pimples resembling those of measles.¹ Mr. H. B. Derr, county agent of Fairfax County, Virginia, states that in an orchard of young Delicious trees the disease is mostly on the northwest side of the trees. The writer has also noted that this is sometimes the case. Some trees appear to be seriously devitalized by the canker, but it is uncertain whether such trees owe their condition to the disease or whether their lack of vigor was a predisposing factor in its development.

At Arlington Farm two French seedlings about 15 years old were the most severely affected, there being scarcely a square inch of the trunks and branches free from the canker. Two Jonathan trees about 20 years old are badly affected at the present time. Three of six 8-year-old Delicious trees are affected, and one 16-year-old Grimes, now removed, was slightly affected.

The writer has received specimens of apple twigs and branches affected with the disease from Kentucky (Valleau, 1923), Virginia (Derr, 1927), and West Virginia (1927). Of commercial varieties, Delicious and Jona-

¹ Rhodes, A. S. Apple measles with special reference to the comparative susceptibility and resistance of apple varieties to this disease in Missouri. *Phytopath.* 14: 289-314. 1924.

than seem to be most susceptible. The writer knows of only two collections of the disease on pear, one from Georgia on the Kieffer variety (Scott, 1908) and one from California (Shear, 1924).

The cause of target canker is not known to the writer, but there are reasons for suspecting that it may be of non-parasitic origin. It is quite possible that it is of the same nature as measles, which it resembles in its early stages. Target canker and measles may be different manifestations of the same disease. The early stages of brown bark spot,² the cause of which is unknown, also resemble those of target canker.

A total of 96 cultures have been made from young cankers at various times. Of these, 54 have been sterile, various species of fungi have developed on 40, and bacteria have developed on 2. The fungi have been chiefly *Physalospora malorum* and species of *Coniothyrium* and *Alternaria*. No inoculations have been made, but inoculation work and further cultural work are contemplated. No fungi have been found fruiting on the cankers, and no mycelium has been found in sections of young cankers.

There is of course a possibility that insect punctures may be responsible, but the following observations made at Arlington Farm incline the writer to the belief that certain conditions of the environment, at present unknown, cause, or at least favor, the development of the disease.

The French seedling apples that were so completely covered with cankers had never been vigorous and were quite evidently growing under conditions unfavorable for their normal development.

The diseased Jonathan apples were affected with crown-gall at the time they were planted and are extremely small and feeble for their age. Other* and more vigorous Jonathan trees of the same age are free or nearly free from the disease.

In a small 8-year-old orchard planted on a slope extending downward from east to west about 120 feet there are six trees of Delicious, two of which are near the upper part of the slope, two about half way up, and two at the bottom of the slope. The last two are much larger than the others, supposedly because of a more bountiful supply of water and nutrient materials. Neither of these trees has more than a slight trace of the disease. Of the other four trees, one is nearly free from the disease but the others have hundreds of cankers. In the writer's notes these diseased trees are referred to as Delicious trees 1, 3, and 4, respectively, and they will be so designated here. The following observations were made on April 14, 1927:

Tree 1. The affected branches are mostly on the west side of the tree, although the branches on the north side also have a few cankers. Certain branches, badly cankered throughout, arise from limbs which otherwise are

² Swingle, D. B., and H. E. Morris. The brown bark spot of fruit trees. Mont. Agr. Exp. Sta. Bul. 146. 1921.

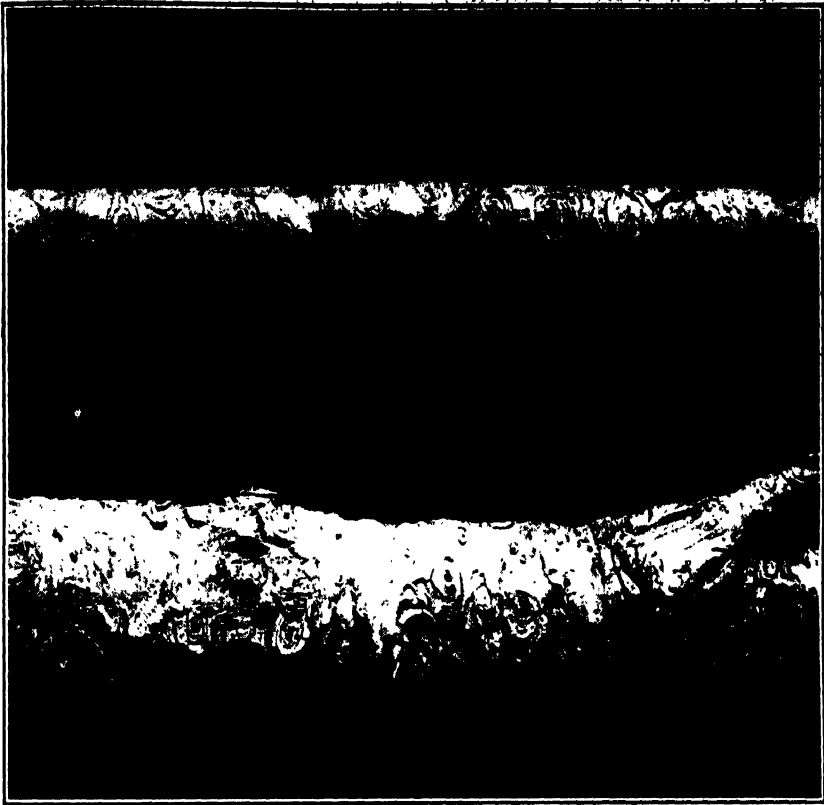


FIG. 1. Target canker of apple. Above, on a three-year old, below, on a six-year old branch of a French seedling. Arlington Farm, Virginia. September 29, 1923.

not affected, the cankers ceasing abruptly at the point where the healthy and diseased branches unite. The tree grew very little last year.

Tree 3. Every limb has some target cankers. The branches on the west side are the most severely affected, those on the north are less affected, those on the east have only a few cankers, while the large and vigorous southernmost limb is almost free. One small twig of this limb is badly affected, the cankers stopping abruptly where the twig joins a larger branch. A small badly cankered branch on the northeast side of the tree is dead. The tree as a whole grew only slightly last year.

Tree 4. Branches on the west and north sides are badly cankered; those on the east and south sides show only occasional cankers. One branch which is near the center of the tree and which forms a fork with the most severely cankered branch has the least cankers of any of the larger branches. Here again the disease ends abruptly at the juncture of the two branches. The tree grew somewhat more vigorously last year than did tree 3.

The relatively small number of cankers on the more vigorous of the Delicious and Jonathan trees, the greater prevalence of cankers on the west and north sides of the trees, the frequent restriction of the cankered areas to certain limbs—which may be almost completely covered by them, and the apparent internal origin of the cankers indicate that the disease is of non-parasitic nature. These observations considered separately lend but slight support to this view, but taken together and considered with the fact that as yet no organism has been consistently isolated from the young cankers they make this view tenable.

As the writer has no experimental data on the subject, he is not able to make any definite recommendations for control. The observation that the more rapidly growing trees are relatively free from the disease suggests that if trees are kept in good growing condition they are not liable to be affected and that the recovery of affected trees may be hastened by employing the usual means for increasing their vigor.

OFFICE OF FRUIT DISEASES,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

STUDIES ON THE CONTROL OF MILLET SMUT¹

L. E. MELCHERS²

Although millet (*Chactochloa italica*) is not a crop of major importance in Kansas, its value ranges between half and three-quarters of a million dollars annually. It is grown in a large number of counties in the state practically every year. The most important disease attacking this crop is the smut caused by *Ustilago crameri* Koern. In 1924 an unusual number of inquiries were made concerning millet smut control. The only known seed treatment at that time was the long-time formaldehyde treatment which required soaking the seed for two hours in a formaldehyde solution. This causes swelling of the seed and necessitates drying before it can be sown. The formaldehyde treatment, although effective, is not wholly satisfactory. In many instances farmers will sow smutted seed rather than treat it with formaldehyde.

Extensive investigations were being conducted on sorghum smut control at the Kansas Agricultural Experiment Station during 1925 and 1926. It seemed desirable to use some of the more promising fungicides on smutted millet seed and determine their effects on the viability of the seed and on smut. Both liquid and dust treatments were used. Table 1 gives the results obtained during 1925 and 1926. In 1925 sowings were made on two separate dates, while in 1926 only one sowing was made. All the seed was artificially smutted, the spore load being much heavier than ordinarily would occur on millet seed. It was desirable to ascertain the limits of efficiency of the several fungicides. Heavy infection was secured in the untreated checks.

The results of two years' data on millet smut control show that:

1. The wet treatments which are most effective for smut control are those with formaldehyde, copper sulphate, and Uspulun.
2. Formaldehyde, either dry or as a mist, should never be used for millet smut control. It seriously injures the germination of the seed.

¹ Contribution No. 269 from the Department of Botany and Plant Pathology. Co-operative investigations between the Kansas Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

² Acknowledgment: the author is indebted to C. O. Johnston and C. H. Ficke for their aid in these studies.

TABLE 1.—Results of seed treatments for the control of millet smut, *Ustilago crameri*, at Manhattan, Kansas, 1925 and 1926

Row no.	Treatment	1925				1926			
		Sown April 22		Sown May 11		Sown May 14			
		Total no. heads*	No. smutted heads	Per-centage smut	Total no. heads*	No. smutted heads	Per-centage smut	Total no. heads	Per-centage smut
1	Formaldehyde 1-320, soak 1 hr.	0	0.0	0	0.0	152	0.0
2	do	0	0.0	0	0.0	126	0.0
3	Formaldehyde 1-320, soak 2 hrs.	0	0.0	0	0.0	147	0.0
4	do	128	5	3.9	0	0.0	139	0.0
5	Check, smutted not treated	135	46	34.0	119	87	74.3	127	32.2
6	Dry formaldehyde, 3 cc. per qt. seed, covered 5 hrs.	no stand	no stand	65	0.0
7	Uspulun 1-400, soak 1 hr.	161	2	1.2	0	0.0	117	1
8	Semesan 1-400, soak 30 min.	75	75	49.3	123	50	40.6	147	6
9	Germisan 1-400, soak 30 min.	134	5	3.7	107	11	10.3	169	3
10	Check, smutted not treated	111	46	41.4	141	59	41.8	107	63
11	Copper sulphate, 1 lb. to 10 gal. (1-80), soak 10 min.	0	0.0	141	4	2.8	174	0
12	Super Kalimat 1-400, soak 15 min.	149	18	12.3	80	3	3.7	102	0
13	Copper carbonate (Cal. Chem. Co.), 2 oz. per bu.	111	6	5.4	0	0.0	112	1
14	Copper carbonate (Cal. Chem. Co.), 4 oz. per bu.	0	0.0	0	0.0	119	0
15	Check, smutted not treated	115	28	24.3	99	42	42.4	131	57
16	Copper carbonate (Cal. Chem. Co.), 6 oz. per bu.	122	3	2.4	0	0.0	104	0
17	Coppercarb., 2 oz. per bu.	139	3	2.1	125	2	1.6	78	1
18	do 4 oz. per bu.	0	0.0	129	3	2.3	109	0
19	do 6 oz. per bu.	0	0.0	157	2	1.2	87	1
20	Check, smutted not treated	104	28	26.9	126	54	42.8	109	47
21	Niagara Dusting Sulphur, 2 oz. per bu.	128	25	19.5	103	12	11.5	103	9
22	do 4 oz. per bu.	119	22	18.4	130	14	10.7	103	3
23	do 6 oz. per bu.	109	6	5.5	74	12	16.2	105	4
24	Bayer dust, 2 oz. per bu.	138	8	5.8	112	13	11.6	124	17
25	Check, smutted not treated	99	23	23.2	35	14	40.0	97	46
26	Sulphodust, 2 oz. per bu.	109	10	9.1	Row killed by chinchbugs	115	21
27	Colloidal copper, 2 oz. per bu.	50	4	8.0	do	do	109	3

* No count was made in rows where smut was entirely absent. The number of heads per row was approximately 110 to 130.

3. The most effective and economical dust is copper carbonate. At least 4 ounces of this dust per bushel of seed are required for the best results.

4. Other dusts, such as various sulphur dusts, Bayer dust, and colloidal copper dust, are not satisfactory for controlling millet smut.

These studies show that the laborious formaldehyde method for millet smut control may be replaced by the more convenient copper carbonate dust method. Not only is copper carbonate effective for the control of sorghum and millet smuts, but it is cheaper and easier to apply than formaldehyde.

KANSAS AGRICULTURAL EXPERIMENT STATION,
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PHYTOPATHOLOGICAL NOTES

Increasing Prevalence of Hypochnus Rot of Apples.—In 1903, Eustace¹ described a rot of apples caused by *Hypochnus* sp. This rot was not reported outside of New York, and there only on Baldwin and Rhode Island Greening apples, where it was consistently associated with and apparently followed scab.

Eustace pointed out the similarities of the macroscopic appearance of *Hypochnus* rot and the pink mold rot caused by *Cephalothecium roseum* and described the differences as follows: "On fruit affected with *Cephalothecium roseum* there is usually a conspicuous white or pinkish growth of the fungus in the center of an affected spot; whereas the new fungus does not show at all conspicuously on the surface of a decayed spot until made to do so by artificial conditions. On fruit, *Cephalothecium roseum* is a very shallow growing fungus, penetrating the tissue not much more than an eighth of an inch, while the new fungus grows much deeper and in its late stage extends to the core." Eustace found that the *Hypochnus* fruited rarely in culture or on apples and indicated the diagnostic value of the clamp connections consistently present in the mycelium.

For the past two years a rot agreeing in essentials with that described by Eustace has been found on Baldwin apples on many of the eastern markets. Specimens have been found at Chicago, Milwaukee, New York, Cleveland, Detroit, Columbus, and Indianapolis. The rot is quite common on New York Baldwins, and it is noteworthy that, while cultures have been made from many of the specimens found, *Cephalothecium roseum* was not isolated in a single instance. This indicates that, at least during the past two years, *Hypochnus* rot has been much more prevalent on the markets than pink mold rot. *Hypochnus* rot has also been found on New York Greenings but less frequently than on Baldwins.

A fungus which has characteristic clamp connections and appears to be *Hypochnus* has recently been isolated from several lots of Winesaps from Yakima and Wenatchee, one lot of Winesaps from Southern Illinois, from Ben Davis and Roxbury Russett apples from New York, from Ben Davis apples from Southern Illinois, and from one lot of Jonathan apples from Idaho.

On Winesaps and Jonathans the lesion looks much like Northwestern anthracnose, in many instances showing a conspicuously tan center with a

¹ Eustace, H. J. Two decays of stored apples. N. Y. (Geneva) Agr. Exp. Sta. Bul. 235. 1903.

brown to black border. The necrotic tissue, however, is somewhat tough and stringy, in contrast to the soft consistency of the true anthracnose or bull's-eye rot lesions as found on the market. On Winesaps in storage the rot is not much in evidence until rather late in the storage season, and has not been found following scab. On Baldwin, Ben Davis, and Rhode Island Greening apples the decay is similar to that on Winesap and Jonathan but is most often found following scab. When infected apples are kept in a moist chamber, the mycelium spreads over the surface of the apples, radiating uniformly from the lesions and forming a characteristic, fine, closely appressed, white mycelial mat.—L. F. BUTLER, Office of Fruit Disease Investigations, Bureau of Plant Industry, Washington, D. C.

Purification of the Virus of Tomato Mosaic.—As a preliminary step in the study of the properties of the virus of tomato mosaic it has seemed highly desirable to free the virus as completely as possible from the various constituents of the plant juice and to obtain the virus in large quantities in a clear and colorless water suspension. This has been accomplished by two methods.

In one method the juice from crushed mosaic plants was filtered through a sintered glass filter and the residue on the filter repeatedly washed with distilled water until the filtrate was colorless. The residue was then suspended in distilled water, and the clear filtrate obtained was proved to contain the virus because young plants inoculated in an insect-free greenhouse became infected.

In the second and more effective method the juice from crushed plants was centrifuged at 35,000 revolutions per minute and the fairly clear supernatant liquid passed through a powdered charcoal filter. The powdered charcoal was then washed with distilled water until the filtrates were clear and colorless, after which the virus was liberated from the charcoal and obtained in a clear and colorless water suspension, which was shown by inoculations to be infectious.—P. H. BREWER, H. R. KRAYBILL, and MAX W. GARDNER, Purdue University, Lafayette, Indiana.

REPORT OF THE ELEVENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held in conjunction with the annual meeting of the Pacific Division of the American Association for the Advancement of Science at the University of Nevada at Reno, and was called to order by President W. S. Ballard on the afternoon of June 23, 1927.

Twelve members were present.

During the business session a motion was passed that the society recommend to the Committee on Nomenclature the changing of the name Western Yellow Tomato Blight to "Tomato Yellows."

OFFICERS OF THE SOCIETY

<i>President</i>	W. S. BALLARD
<i>Vice-President</i> ..	W. G. MILBRATH
<i>Secretary-Treasurer</i> ..	B. A. RUDOLPH
<i>Councilor</i>	W. T. HORNE

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

Notes on fruit decays of the feijoa (Feijoa sellowiana Berg).—W. T. HORNE.

The feijoa is an ornamental shrub which has fruit for home use and local markets. It possesses unique qualities, and its season (November) gives it an interesting place following the summer fruits.

Studies carried on principally with fruit from the Citrus Experiment Station, Riverside, were the basis for the following conclusions. Gray mold (*Botrytis cinerea* Pers.) appears to be the most active and common cause of spoilage. The decay produced is moderately tough and becomes somewhat dry; the fruit is rather quickly invaded and characteristic flavors destroyed. In cool dry air infected fruit merely withers.

Apple green mold, *Penicillium expansum* Lk., is the next most important agent of decay. The blue and green molds of citrus were not found occurring naturally on feijoa, and as a result of inoculation developed only poorly. Anthracnose or citrus wither tip fungus, *Colletotrichum gloeosporioides* Penz., was found in one case. Numerous other molds, including *Alternaria*, develop abundantly on over ripe fruit.

The treatment of lime-induced chlorosis in fruit trees.—J. P. BENNETT.

Effective treatment of chlorotic pear trees growing in a calcareous soil has been accomplished by placing solid ferric citrate in holes bored into the trees below the graft-union and below the ground level. Holes 1 centimeter in diameter and 3 to 5 centimeters in depth were bored at intervals of 8 to 10 centimeters around the main

root, one-half gram of the powdered salt placed in each hole, and the holes closed with grafting wax. The treatment was most effective when applied during the dormant season; during the active season, injury to the leaves frequently resulted. Complete greening of all leaves in the season following the treatment has occurred in 90 per cent of the 5,000 trees treated; incomplete greening in the remainder is attributed to poor distribution of the iron salt as a result of too small a dosage. The effect of the treatment has lasted for two years. The cost of treatment varied between two and ten cents per tree, according to size, in trees from three to thirty years old. Similar results have been obtained with chlorotic prune and walnut trees. Effective treatments have also been carried out in the same manner with ferrous tartrate, ferrous sulphate, ferric malate, and other soluble iron salts.

Celery experiments.—T. E. RAWLINS.

The addition of 2½ per cent soil from celery roots to fertile potting compost was found to treble the early growth of celery plants in such compost. Those plants to which no celery soil was added were stunted, chlorotic, and had necrotic leaf margins, while those to which celery soil was added had a very healthy appearance.

Further experiments to determine whether this increased growth is due to the celery mycorrhiza are in progress.

Inoculation experiments with western yellow tomato blight in relation to environmental conditions.—MICHAEL SHAPOVALOV.

Experiments were carried on which prove conclusively that the curly top virus, when introduced in the tomato plant by means of viruliferous *Eutettix tenella* Baker, may under specific environmental conditions produce a pathological phenomenon indistinguishable from that known as western yellow blight of tomatoes. It was practically impossible to attain the complex of field symptoms under greenhouse conditions, particularly with caged plants, but perfect specimens of blight were produced with tomatoes grown outdoors unprotected or in light muslin cages.

Shading retards the progress of the disease and partly prevents it. Under heavy shading certain field characteristics of blight are lacking, while symptoms of curly top are more conspicuous. Light and humidity appear to be the most important single factors in the development of the complete field symptomology. High temperature may shorten the incubation period but in the absence of proper light and humidity conditions cannot bring about the characteristic blight complex. Certain other factors, such as the age of the plant, may either strengthen the resistance of the host or weaken it.

It is suggested that the name "western yellow tomato blight" be changed to "tomato yellows": (a) for the sake of brevity; (b) to conform with facts, and (c) to include similar diseases of other crops.

*Relation of temperature to growth of *Penicillium italicum* and *P. digitatum* and to citrus fruit decay produced by these fungi.*—H. S. FAWCETT and W. R. BARGER.

On culture media as well as on orange fruits, *P. italicum* and *P. digitatum* both appeared to have nearly the same optimum temperature for development. The difference, however, between the rate of growth at the optimum and the rates at higher and lower temperatures was greater in the case of *P. digitatum* than *P. italicum*.

In most cases the rate of development of decay was much more rapid at the stem end of the orange fruits than at the styler end. The percentage of decay due to

injuries near the stem end was also greater in most cases than that due to equal injuries near the stylar end.

In lots of fruit inoculated with *P. italicum* and held respectively at 66.8, 74.8, and 80.6° F., nearly all fruits started to decay in 4 days, while lots held at 86 and 57.5° F. were in similar condition in 8 days, and lots held at 50° F. in similar condition in 12 days.

In lots inoculated with *P. digitatum*, much the same general relation of temperature to time and percentage of decay was noted, except that apparently the temperatures above and below the optimum had a greater relative inhibiting effect than in lots inoculated with *P. italicum*.

The enzymes of Pythiacystis citrophthora Sm. and Sm.—I. JOS. KLOTZ.

In a survey of the enzymes of the mycelium of *Pythiacystis citrophthora*, the following were tested for: osterases, cellulase, cytase, pectinase (pectase), inulase, diastase, raffinase, invertase, lactase, maltase, emulsin (amygdalase, salicinase, arbutinase), glucosidases that attack hesperidin and phloridzin, tannase, amidases (deaminases, deamidases, urease), histozyme, proteases, rennet, zymase, peroxidase, oxidase, catalase, reductase, and tyrosinase.

Very positive evidence was obtained for the presence of some of the lower esterases, for diastase, invertase, maltase, emulsin, phloridzinase, asparaginase, urease, peroxidase, and catalase; less evidence is forthcoming for the presence of cytase, lactase, hesperidinase; there was very slight indication of the presence of inulase, pectinase, protease and glycolase; and for the remaining enzymes sought the results were entirely negative.

The necessity for check determinations on both the active and deactivated enzyme material is emphasized, and a more accurate method of calculation given.

The diastase of this fungus attacks gelatinized starch vigorously, but starch suspended in cold water only feebly.

Although urea solution of the strength tried could not be used by the fungus, the enzyme powder gave a strong urease reaction.

Ecological studies of curly-top of sugar beets.—WALTER CARTER.

High light intensity, temperature, and evaporation appear to favor the development of severe curly-top symptoms. These same factors apparently affect the number of positive cases obtained.

Experiments on the control of the external environment were conducted, using various pigments. The transmission capacity of sprayed and unsprayed leaves was measured with a Kimball pyrliometer. Significant differences in the yield of beets sprayed with pigments were obtained.

Dehulling barley seed with sulfuric acid to induce infection with covered smut (Ustilago hordei).—FRED N. BRIGGS.

Experiments conducted by the writer in the greenhouse at Berkeley showed that barley seeds dehulled by sulfuric acid and inoculated with covered smut showed percentages of infected plants of the same magnitude as inoculated hand-dehulled seeds. The seeds were soaked in a quantity of acid from two to three times the volume of seed and then washed for 20 minutes under a faucet fitted with a shower or rose spray. Tennessee Winter barley grown from seed treated with various concentrations of sulfuric acid and seeded in the field in the fall of 1926 at Davis, California, showed the following percentages of infection with covered smut:

Concentration of acid	Duration of treatment	Plants			
		Produced		Smutted	
		Percentage	Number	Number	Percentage
Check	No treatment	80.0	60	5	8.3
do	Dehulled by hand	48.0	36	20	55.6
Conc.	5 min.	64.0	48	14	29.2
do	10 do	30.7	23	11	47.8
90 per cent	15 min.	18.7	14	9	64.3
do	20 do	6.7	5	1	20.0
80 per cent	25 min.	38.7	29	13	44.8
do	35 do	4.0	3	1	33.3
70 per cent	30 min.	69.3	52	11	21.2
do	40 do	74.7	56	29	51.8
do	50 do	45.3	34	23	67.6
60 per cent	1½ hrs.	69.3	52	22	42.3
do	2 do	48.0	36	19	52.8
do	2½ do	56.0	42	27	64.3
50 per cent	4 hrs.	36.0	27	14	51.9
do	5 do	24.0	18	14	77.8
do	6 do	16.0	12	9	75.0
40 per cent	6 hrs.	58.7	44	25	56.8
do	7 do	58.7	44	24	54.0
do	8 do	61.3	46	35	76.1
30 per cent	24 hrs.	17.3	13	9	69.2

(Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Agronomy Division of the California Agricultural Experiment Station).

A cytological study of Puccinia coronata Cda. on Banner and Cowra 35 oats.—MABEL L. RUTTLE, and W. P. FRASER.

The development of uredinia and telia of crown rust (*Puccinia coronata* Cda.) on Banner oats (susceptible) and Cowra 35 oats (variable in resistance) was studied cytologically. The formation of appressoria and entry take place in the same fashion on both. Appressoria are sometimes lobed. The substomatal vesicle is first a rounded sac which changes later into a canoe-shaped body from each end of which a slender infecting hypha is given off. The binucleate haustorium mother-cell is about 12 μ long. Haustoria on Banner are unbranched and uninucleate, attain a maximum length of 30 μ , function for a few days, are drained, and then appear as empty shells. Haustoria on Cowra 35 attain a maximum length of 18 μ , function briefly if at all, are seldom drained, and are frequently killed. In infected plants of Banner the host tissues give evidence of being stimulated by the fungus and show heightened turgor. The nuclei move over to the haustoria, increase somewhat in size, then contract and die. On Cowra 35, attacked cells often collapse and die, and adjoining cells show increased turgor, impoverishment, and enlargement of nuclei. Numerous yellowish-brown, rounded intracellular bodies varying in diameter from 3 to 18 μ are found in old infections and uninfected tissues dying of old age. Paraphyses are sometimes present at the margin of the uredinium.

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THE FUSARIUM DISEASE (WILT) OF COTTON AND ITS CONTROL¹

T E W F I K F A H M Y

INTRODUCTION

Cotton is the most important crop in Egypt, on which the prosperity of the country depends. In 1925 the total area in cotton was 1,924,382 feddans,² of which 1,128,946 feddans were in Sakel and 795,436 in Ashmouni and other varieties.

The Sakel crop for that year was estimated at 3,779,990 kantars, and the Ashmouni and other varieties at 4,080,939 kantars,³ making a total of 7,860,929 kantars.

The average price of Sakel per kantar in 1925 was \$35.78, and for the Ashmouni \$25.06, making a total approximate value of \$135,258,000 for the Sakel and \$20,453,000 for the Ashmouni and other varieties.

So far the varieties most affected by *Fusarium* disease are those producing the long-staple cotton, of which Sakel is the principal and the one most grown in the Delta. Ashmouni and Zagora, two short-staple varieties grown mostly in Upper Egypt, are immune to this disease.

HISTORY

Atkinson (2) in 1882 was the first to investigate *Fusarium* disease in the United States. The fungus was isolated and described as a new species under the name *Fusarium vasinfectum*.

Erwin F. Smith (11) in 1899 described what he thought to be the ascogenous stage of the *Fusarium* of cotton wilt and called it *Neocosmospora vasinfecta* (Atk.) Syn. *Fusarium vasinfectum* Atk. He described it as follows: "Probably also on okra. Parasitism not proved. Genetic connection of various spore forms not proved. Chlamydospores not observed."

W. A. Orton (10) in 1900 produced the wilt disease "in healthy cotton plants by inoculating the soil in which they grew with pure cultures of conidial stages of *Neocosmospora vasinfecta*."

¹ A detailed bulletin by the author is in the press. The author is indebted to Dr. E. C. Stakman for his assistance in preparing the manuscript.

² The feddan is 4,200 square meters.

³ The kantar (unginned) is 141 kilograms.

Victor Mosseri (9) in 1902 was the first to diagnose this disease in Egypt and attributed it to *Neocosmospora vasinfecta* (Atk.) Smith.

Higgins (8) in 1909 in North Carolina and Butler (4) in 1910 in India have thrown some doubt on the parasitism of *Neocosmospora* and on its genetic connection with the wilt-producing *Fusaria*.

Other workers in the United States and elsewhere published short studies on this disease. The most important are those of Gilbert (7) in the United States, Ajrekar and Bal (1) and Butler (5) in India, and Briton-Jones (3) in Egypt.

DISTRIBUTION

A survey made during the cotton-growing seasons of 1924 and 1925 has shown that the disease is prevalent in the fertile parts of the Delta and absent in Upper Egypt.

In Lower Egypt the disease was found to be severe in certain localities, less so in some, and absent, as far as known, in other localities.

In Upper Egypt, where Ashmouni and Zagora, two varieties resistant to this disease, are grown, a peculiar localized discoloration has been observed within the central cylinder of certain plants of these varieties. This discoloration is much deeper in color than the characteristic one found in diseased susceptible varieties, such as Sakel cotton, and is localized in a way not found in typically diseased plants. From this discolored zone a species of *Fusarium* was isolated, but so far has not proved to be parasitic on Sakel or Ashmouni in spite of numerous inoculations. Besides, Sakel cotton—

TABLE 1.—The occurrence of the *Fusarium* disease of cotton in Lower Egypt

Province	District	Severity of attack	Province	District	Severity of attack
Gharbia	Tanta	Very severe	Menoufia	Ashmoun	Very severe
	Kafr El Zayat	do		Shibin El Kom	do
	Dessouk	Severe		Tala	do
	Zifta	do		Quesna	Severe
	Santa	do	Behera	Menouf	do
	Mahala El Kobra	do		Damanhur	Very severe
	Talkha	do		Abu Homos	do
	Kafr El Sheikh	do		Delingat	do
	Sherbin	Slight		Kom Hamada	do
	Foa	do		Teh El Baroud	Severe
Dakahlia	Belkas	Very slight	Sharqia	Shubrakhiet	do
	Simbilawin	Very severe		Belbis	Slight
	Aga	do		Hehia	do
	Mit Ghamr	do		Fakous	do
	Mansura	Slight	Qaliubia	Toukh	Slight
	Faraskour	Very slight		Qualuib	Very slight

which is very susceptible to this disease—was grown in fields where Ashmouni and Zagora cotton plants were previously found locally discolored, but in no case did Sakel become infected. It is therefore logical to conclude that at present this disease is absent in Upper Egypt and that this peculiar localized discoloration found in Ashmouni and Zagora is possibly due to one or more non-parasitic soil *Fusaria* which may enter the cotton plant through some mechanical or other injury, but which are unable to develop within the living plant tissue beyond a localized portion.

Table 1 gives the localities in Egypt where the disease was observed, as well as the severity of the attack.

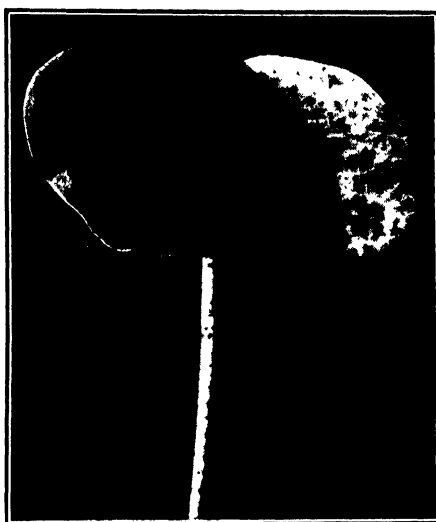


FIG. 1. Early mosaic symptoms of *Fusarium* disease on cotton leaf.

ECONOMIC IMPORTANCE

The parasite attacks principally long-staple varieties of cotton. The disease is so severe in some parts of the Delta that many cultivators have given up growing Sakel cotton and have substituted short-staple varieties resistant to *Fusarium*. The conditions in Lower Egypt are most suitable for growing Sakel, the most important of the Egyptian long-staple cottons. It would be, indeed, a great loss to the country, if, as a result of *Fusarium* disease, the growing of this long-staple variety had to be abandoned.

At present, Egypt produces the bulk of the long-staple cotton of the world. Much of the prosperity of the country depends on this fact. Since the great war, many other countries have started cotton growing on a large scale, but they will probably produce cotton of a shorter staple than the Egyptian long-staple varieties. It seems to the author, therefore, that to maintain the present prosperity of this country the production of long-staple

cotton must be kept up. To realize this, the Fusarium disease must be controlled in a way that will not interfere with the production.

Happily this work has led to the isolation of four strains of Sakel which are perfectly resistant to this disease and of good long-staple quality. This matter will be considered later in this article.

DESCRIPTION OF THE DISEASE

The name "wilt" for the Fusarium disease may suggest that the wilting of the diseased plant is a necessary symptom. Under Egyptian conditions such is not the case. Infected plants may appear externally healthy, and

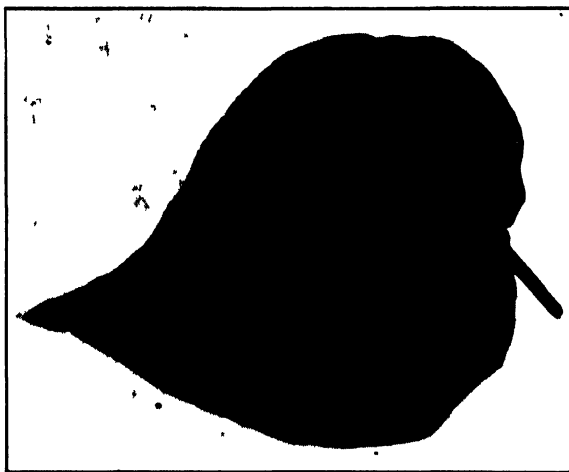


FIG. 2. Later symptoms of Fusarium disease of cotton, in which the entire leaf surface is mosaic.

yet on examination of the roots the characteristic discoloration can be easily observed in the tissue of the central cylinder. For this reason "Fusarium disease" is suggested as a name for the disease as it occurs in Egypt.

External Symptoms: the mosaic symptoms of the leaf. The very characteristic symptom is the appearance of a yellowish network on the leaves. This mosaic, as it is called, starts generally in one corner of the leaf (Fig. 1) and extends until it may cover the whole leaf surface (Fig. 2). It is apparent on both sides of the leaf, but mostly on the upper surface. The affected part of the leaf may or may not dry up in the former case; it turns light brown.

This mosaic symptom appears more frequently in the seedling stage than in the adult plants. It is of very frequent occurrence in susceptible cotton seedlings grown in infected soil in pots. Its frequent occurrence seems to be associated with abundant soil moisture.

Although the mosaic has been observed as a sure sign of the disease, its association with diseased plants is not constant, and for this reason its absence does not indicate freedom from disease.

External Symptoms: the death of the stem. The stem of a diseased plant may die, starting at the growing point and proceeding downwards. The whole plant may die or may survive by giving rise to new shoots from the lower portion of the stem (Fig. 3, A). The newly formed shoots may



FIG. 3. A. Cotton plant in which the growing point has been killed by *Fusarium*. New shoots have grown out from the lower portion of the stem. B. Infected cotton plant which is very much dwarfed and has produced numerous basal branches.

in turn show signs of the disease. Later, however, the recovered plants may be dwarfed, with much basal branching and poor productive capacity (Fig. 3, B).

Internal Symptoms. The roots of a diseased plant which has passed the seedling stage always show a characteristic discoloration on being cut longitudinally. The discolored tissue is dark olive green and is arranged in irregular "bundles of streaks" (Fig. 4). This discoloration, which is in the central cylinder of the plant, may extend from the root system upwards. It may reach the growing point and even the boll-stalk. Its presence in the boll-stalk as a possible source of infection of the seed is discussed later.

THE CAUSAL ORGANISM

The Parasite within the Host. When hand sections of the roots of an infected plant are examined microscopically, hyphae of the parasite are found within some of the xylem vessels in the discolored tissue. These hyphae may be so crowded together as to plug some of the vessels or may be so sparse that only a few can be observed (Fig. 5).

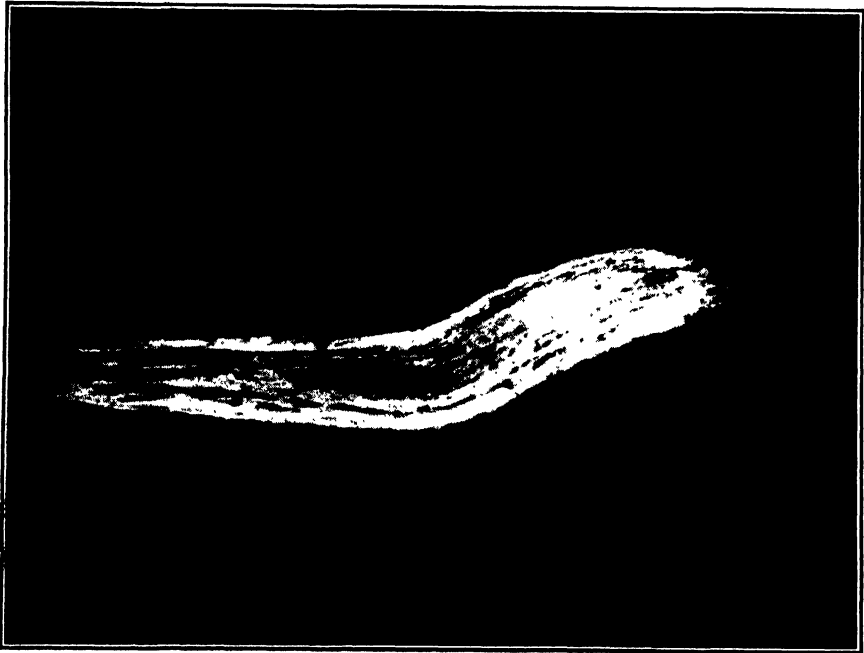


FIG. 4. Root of a cotton plant split to show the discolored areas characteristic of Fusarium disease.

The hyphae may be creamy white or of a yellowish tinge as if externally coated with a pale yellow gum-colored substance. The walls of the xylem vessels may also be stained a deeper yellow. The same yellowish coloring matter may also fill some of the smaller cells near the invaded xylem cells. Other cells may also be filled with a very dark gum-like substance. Tyloses

have also been observed. So far no spores have been found within the invaded cells.

During the life of the plant the parasite seems to limit its invasion to the xylem vessels; at the death of the host plant, however, and if it is kept under moist conditions, the parasite appears on the surface of the infected portion as tufts of mycelium bearing myriads of spores.

Isolations. The fungus is easily isolated from diseased plants by placing short lengths of peeled infected roots or stems under aseptic conditions on any suitable medium. The fungus, which is a species of *Fusarium*, appears generally within a week as white mycelial tufts, either from one or both of the cut ends, or from the peeled surface. At first the tufts consist of hyphae with few microconidia, later macroconidia are produced, and then chlamydospores.

Parasitism. Isolations were made from diseased plants obtained from infected fields in the different provinces of Lower Egypt, where the disease is known to be severe. The fungus was transferred on acid potato plug, on which it grows vigorously; after 10 days' incubation at 30° C. these cultures were mixed with a weighed amount of soil known to be free from *Fusarium*, and Sakel and Ashmouni cotton were sown in it. The fungus in all cases proved to be parasitic on Sakel, while Ashmouni cotton was immune. The parasite was re-isolated from the diseased plants and proved again to be parasitic on Sakel.

The Morphological and Cultural Characters of the Fungus. A single spore culture was obtained and tested for its pathogenicity. The culture developed normally, and produced all forms of spores, microconidia, macroconidia and chlamydospores. It was examined after 40 days at 30° C.

Microconidia: 0-3 septate, typically 0 and 1 septate, majority ellipsoidal, sometimes slightly curved, few globose to pear-shaped, rarely comma-shaped.

Macroconidia: 0-5 septate, typically 3 and 4 septate, sickle-shaped to straight, gradually attenuate more towards the apex, pedicellate.

Chlamydospores: Subglobose to spherical, generally smooth and thick-coated; terminal, 0-1 septate, formed on or in mycelium; intercalary, single or in chains, rarely in clusters. In old cultures and under reduced aeration, conidial chlamydospores develop, mostly in macroconidia.

Mycelium: Hyphae copious, slightly branched, some very granular, varying greatly in diameter (2.5 to 5 μ). Septation varying in size. The hyphae appear colorless under the microscope, but in mass, when grown on acid potato plug, the mycelium is not dead white but has a very faint bluish hue and is cottony in appearance. Anastomosis occurs, but is rather rare.

No sclerotia have been observed up to now.

Spore germination: At room temperature (25° C.) microconidia and macroconidia germinate readily in water. Some of the chlamydospores germinate while others do not. The drop cultures were kept under observation for one week.

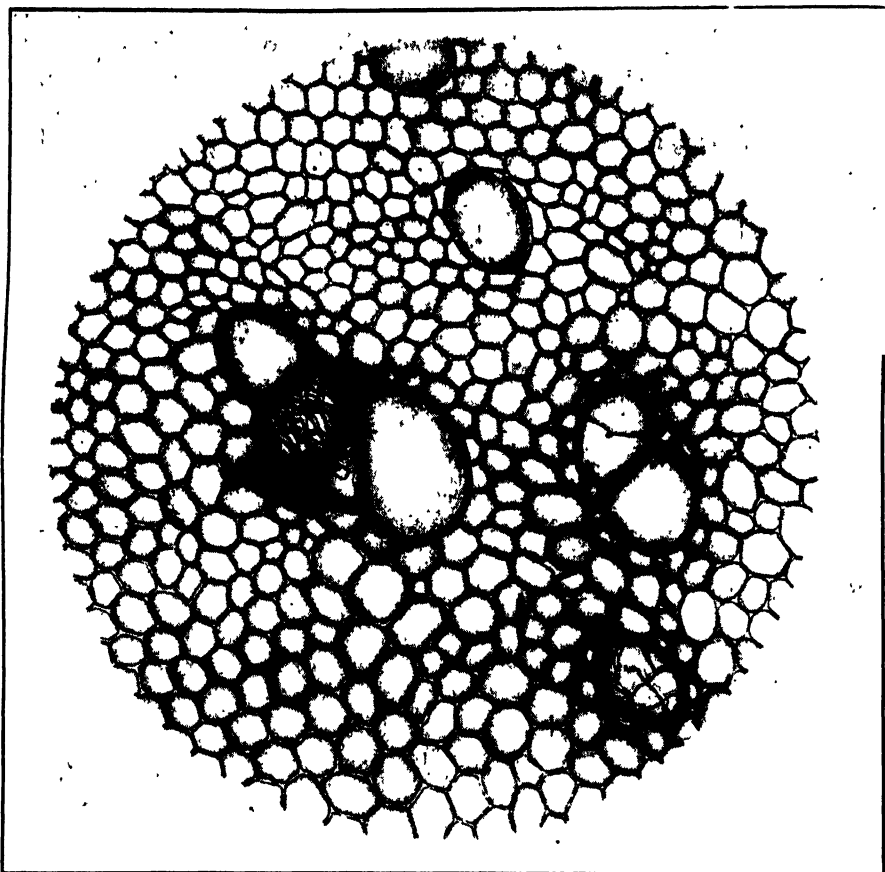


FIG. 5. Cross section of a cotton root showing the hyphae of *Fusarium* in the xylem vessels.

The Growth of the Fungus on Different Media. On standard liquid media⁴ the fungus grows best on those containing 3 per cent glucose: when the same percentage of saccharose is substituted, growth is slightly less.

If 1.5 per cent citric acid is used instead of either of the sugars, there is less mycelial growth but a great development of chlamydospores. On

⁴ The standard medium, as used by Butler (4), was made up as follows:

Ammonium nitrate	10 gms.
Potassium phosphate	5 gms.
Magnesium sulphate	2.5 gms.
Distilled water	1000 cc.

standard medium, to which sodium carbonate has been added, growth is very poor.

On solid media the fungus grows best on acid potato plug (lactic acid).

The Relation between Host and Parasite. The author, in a previous paper (6), studied the production by *Fusarium solani* (Mart) Sacc. of a toxic substance capable of causing wilting in plants. The excretory substance of the Egyptian *Fusarium* of cotton was similarly studied. It was found that when the fungus was grown on modified Richards' solution a staling substance was formed which was capable of causing cotton seedlings to wilt when these were placed in the filtrate of the culture. This staling substance increased with the age of the culture. The alkalinity of the filtrate of the culture also increased with the age.

It is possible that the parasite within its host is capable of producing similar staling substances which may under certain conditions cause the infected plant to wilt.

The Temperature Relation. The optimum temperature for the growth of the fungus on acid potato plugs was found to be between 30° and 35° C.

The incubation period, the time required for the mosaic symptom to appear after sowing susceptible cotton seed in infested soil, varies with the average day temperature. Sakel cotton was grown in uniformly infested pots in February, April, June, July, August and October. Table 2 gives the results.

TABLE 2.—*The effect of the average day temperature on length of incubation period with cotton plants grown in Fusarium infected soil*

Date of sowing, 1924	Average day temperature during growth period in degrees C.	No. days required for mosaic to appear after sowing
Feb. 2	15.9	58
Apr. 18	23.7	14
June 18	26.1	14
July 23	26.9	12
Aug. 28	25.0	14
Oct. 20	20.6	22

It is worth noting that under field conditions, when cotton is sown in March, the first symptoms appear from 60 to 90 days after sowing.

SUSCEPTIBILITY OF COTTON VARIETIES

Egyptian Varieties. The different varieties of Egyptian cotton vary in their susceptibility to this disease: Sakel cotton is the most susceptible,

while Ashmouni and Zagora (a selected strain from Ashmouni) are immune. Table 3 gives the average infection obtained in a number of trials with several Egyptian varieties. The estimation of susceptibility was calculated on

TABLE 3.—*The susceptibility of Egyptian varieties of cotton to Fusarium disease*

Variety	No. of plants			Percentage	Variety	No. of plants			Percentage
	Total	Healthy	Diseased			Total	Healthy	Diseased	
Sakellaridis ...	88	3	85	96	Casuli	131	103	28	21
Garofalou	80	6	74	92	Affi	44	35	9	20
310	189	20	137	73	Toudri	282	236	46	16
Assili	244	111	133	54	Pilion	307	266	41	13
Nahda	60	30	30	50	Fathi	194	181	13	6
Nubari	136	104	42	30	Ashmouni ...	489	489	0	0
Abbassi	87	54	23	26	Zagora	259	259	0	0

the basis of the number of plants which were found typically discolored in the central cylinder of their roots when two months old, plus the number of plants which had died at an early age from the disease. The parasite was isolated from all of these.

Susceptible Age of Host. Infection is possible at any time during the life of a susceptible cotton plant as long as its root comes in contact with the parasite. The seedling stage is, however, the period when injury is most likely to be fatal.

During the warmer part of the year, when seedlings are grown in heavily infected soil, the parasite produces a distinct brick-colored zone at the hypocotyl, somewhat similar to that caused by sore-shin. The seedlings are girdled at that point, thus causing the plants to collapse and gradually dry up.

Other Hosts. The Egyptian *Fusarium* parasite of cotton is specialized in its parasitism not only for cotton, but for certain strains of cotton.

The Mode of Infection. The actual mode of infection is under study. Investigation has shown, however, that the parasite is able to enter the root system of its host unaided by other soil organisms. When injected hypodermically into the stem of a healthy, but susceptible cotton plant, it is able to develop and produce the typical discoloration in the central cylinder at the part injected. When, however, immune cotton plants are similarly injected, the parasite is unable to develop, but produces a localized discolored portion not typical of the disease. In both cases, however, the parasite was reisolated and proved to be parasitic.

Is the disease seed-borne? Under Egyptian conditions up to now, the pathogene does not seem to be seed-borne. Trials over two years were carried out. Seed from heavily infected plants were grown in sterile soil. The seed were divided into two lots; one lot was externally sterilized by delinting in concentrated sulphuric acid, the other sown without disinfecting. In both cases none of the 145 plants grown and allowed to mature developed any external or internal symptoms of the disease. The author also failed to isolate the parasite from the seed of heavily infected plants, which were treated as above. This investigation is being continued.

THE RELATION BETWEEN THE DISEASE AND THE SOIL

The infective capacity of the soil was found to vary within the same field. The disease first appears in the field in patches. With the frequency of growing susceptible cotton the patches increase in area until finally the whole field may become involved. Within these patches the cotton plants die at an early age. In the immediate area surrounding the patches, plants of the same susceptible variety show no definite, apparent signs of the disease, yet when they are pulled up and their roots slit, the characteristic discoloration is found in the majority.

In less infective fields with susceptible varieties of cotton the plants may appear at first sight as healthy. However, when their roots are examined, many are found to be typically infected.

Investigations carried out at Giza have shown that the different degrees of infective capacity of the soil vary with the amount of infecting matter present either through its accumulation year after year, due to the growing of susceptible cotton, or by the addition of different amounts of the organism in culture.

Type of Soil. The soil found infected most frequently is that of heavy texture, contrary to what occurs in the United States where wilt is most prevalent in light soil.

In the Delta the disease is most severe in the heavy fertile land. Experimentally, the attack was more severe with the addition of organic manure to infected land.

Investigation has shown that the fungus may be found as deeply buried in infected fields as one meter and perhaps more. The parasite is probably carried into the deeper layers with the growth of infected roots of the host. Infection is most severe, however, in the first 40 centimeters below the surface.

Observations over a number of years have suggested that in the case of the well distributed soil infection, similar to that which occurs under natural field conditions, the plant is subjected to many repeated infections, as its new roots grow into the soil containing the parasite.

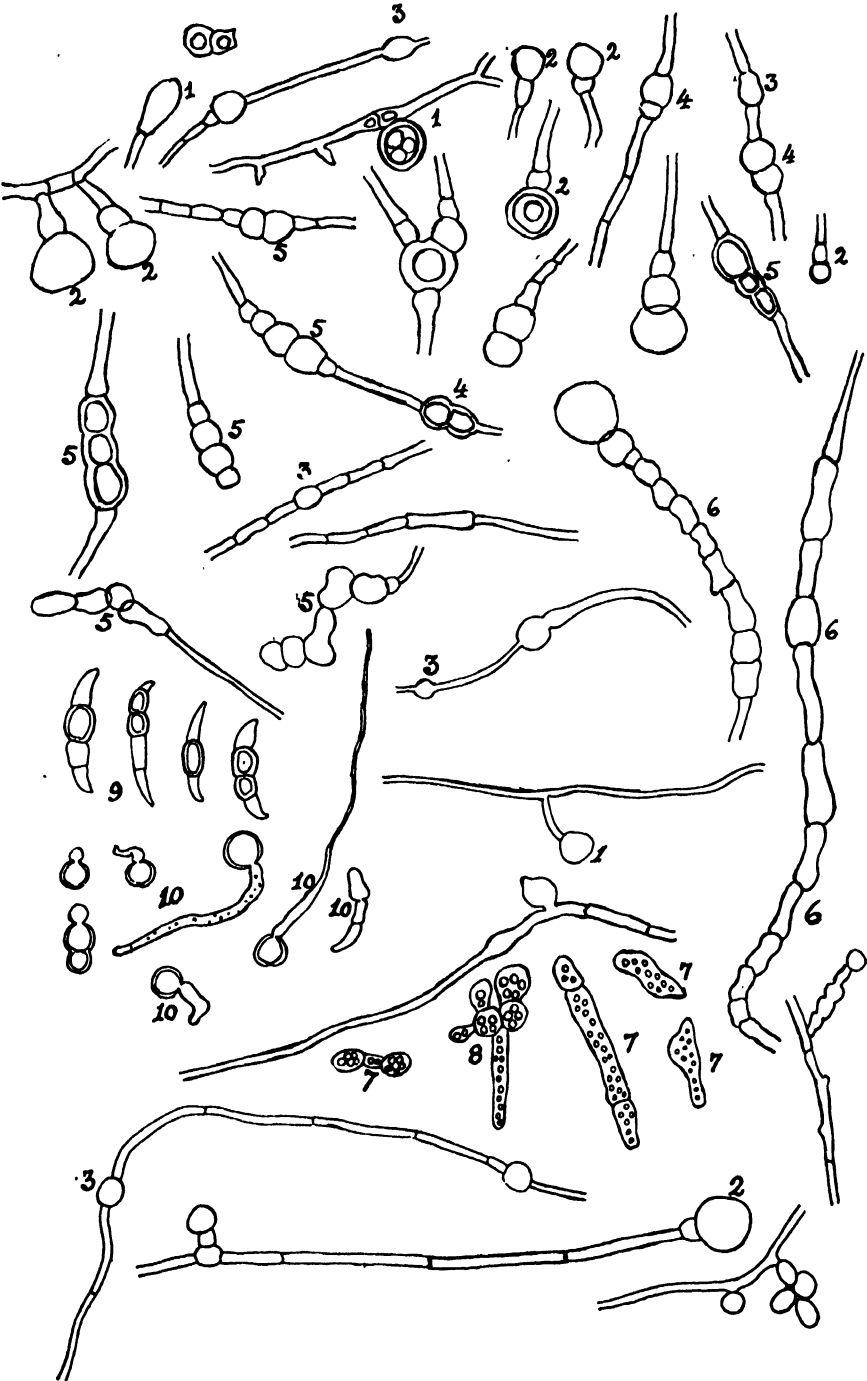


FIG. 6. Various stages of the Egyptian *Fusarium*.

However, in case the soil is artificially infected only 20 or 30 centimeters below the surface, many plants are subjected to attack only in their early stages and later the roots grow into the deeper clean soil layers. Many plants arrest the spread of the parasite within their tissues by forming a gummy substance about the local infections.

CROP ROTATION AND SEVERITY OF DISEASE

A rotation experiment started at Gemaiza farm in 1922 has shown that up to 1926 the severity of disease increases with the frequency of susceptible cotton crops.

On six plots planted with Sakel cotton in 1923, 1924, 1925 and 1926, the average percentage of infected plants in 1926 was 74.5.

On six plots planted with Sakel cotton in alternate years, 1923 and 1925, the average percentage of infected plants in 1925 was 25. On six similar plots planted with the same variety in 1924 and 1926 the average percentage of infected plants was 33.

When Sakel cotton was grown every third year, the average percentage of infected plants was 10 on one series of six plots and 23 in another series of six plots.

THE RELATION BETWEEN THE EGYPTIAN *FUSARIUM*, THE INDIAN *FUSARIUM*, AND *FUSARIUM VASINFECTUM*

To attempt to identify the Egyptian *Fusarium*, it was necessary to compare it with the Indian and American fungi producing wilt diseases in the respective countries.

The morphological differences between the microconidia and macroconidia of these three fungi is too small to be of any comparative value. There are, however, some differences in the morphology of the chlamydospores, substratum coloration, and cultural characters of these respective fungi.

Chlamydospores. The three fungi were grown under identical conditions on acid potato plug and examined after 40 days' incubation at 30° C.

In the case of the Egyptian *Fusarium* there is a tendency for the chlamydospores to become smaller towards one or both extremities of the chain in which they are borne. Dumb-bell-shaped chlamydospores often are produced (Fig. 6).

In the case of the Indian *Fusarium*, the basal cell of the 1-septate terminal chlamydospore is rectangular in shape and definitely constricted at the middle (Fig. 7).

The American *Fusarium* has a peculiar knotted appearance owing to the fact that some of its hyphae contain spherical to globose non-septate inter-

calary chlamydospores and that there is variation in the size of the chlamydospores in some of the chains (Fig. 8).

Substratum Coloration. The substratum coloration of the Egyptian *Fusarium* is very distinct from the Indian and American *Fusaria*.

Table 4 gives the different substratum colorations produced by the respective *Fusaria*.

TABLE 4.—*The substratum coloration produced on, rice and on oatmeal agar by the Egyptian, Indian, and American strains of cotton Fusarium*

Culture of <i>Fusarium</i>	Medium	Substratum coloration
Egyptian	Rice	Brilliant yellow with light cobalt violet in parts.
Indian	do	Light cobalt violet, madder lake in patches, mineral violet ring.
American	do	A ring of deep mineral violet.
Egyptian	Oatmeal agar	Traces of permanent mauve of different shades.
Indian	do	Combination of cobalt violet and mauve.
American	do	Combination of deep mineral violet and light mauve.

Cultural characters. There are only slight differences in the three fungi as to their cultural characters. The American *Fusarium* grows better on some media than the other two. Under reduced aeration and in old cultures, the macroconidia and microconidia of the Egyptian and Indian *Fusaria* form conidial chlamydospores. In the American strain, however, up to now no such chlamydospores have been observed.

The parasitism of the three fungi. The Egyptian *Fusarium* is a virulent parasite on the long-staple Egyptian cotton and the Sea-Island varieties. It also is capable of attacking some of the Indian varieties.

The Indian *Fusarium* is non-parasitic on both the American and the Egyptian varieties, but parasitic on some of the Indian varieties.

The American *Fusarium* is a very weak parasite on the Egyptian varieties, non-parasitic on the Indian varieties tried, but parasitic on some of the American cotton varieties.

The above results were obtained after a series of trials carried out at Giza over a number of years. It is possible, however, that soil and climatic conditions at Giza may not be so favorable for the attack of the Indian and American parasites as the conditions prevalent in the respective countries where they produce the disease. Therefore the above results must be taken with certain reserve.

Moreover, in Egypt this disease is prevalent in heavy soil independently of the attack of *Heterodera radicola*, while in the United States it occurs in light soil and is often associated with the root-knot disease.

The writer, in the light of the above description and behavior of the three fungi, considers that the Egyptian *Fusarium* of cotton is distinct from the American and Indian *Fusaria* producing the same disease in the United

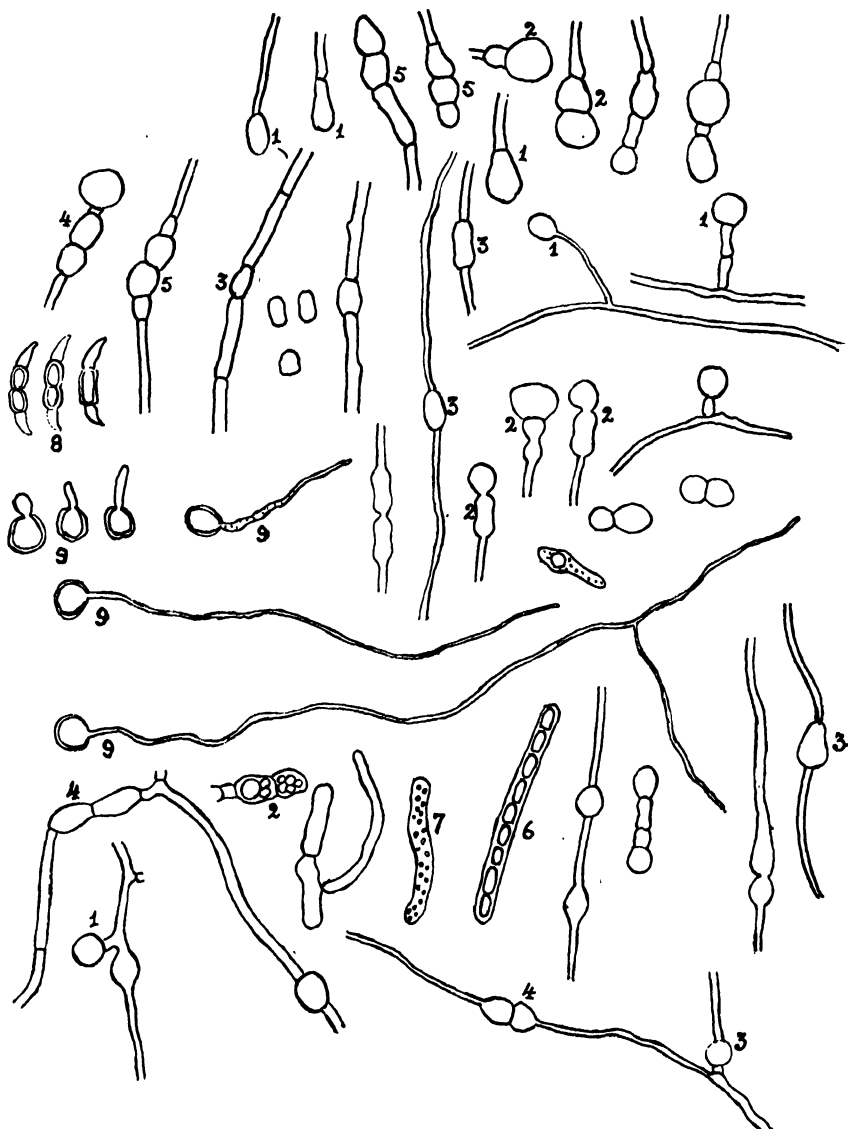


FIG. 7. Various stages of the Indian *Fusarium*.

States and India, and suggests calling this fungus, at any rate until further studies are carried out, *Fusarium vasinfectum* var. *egyptiacum*.

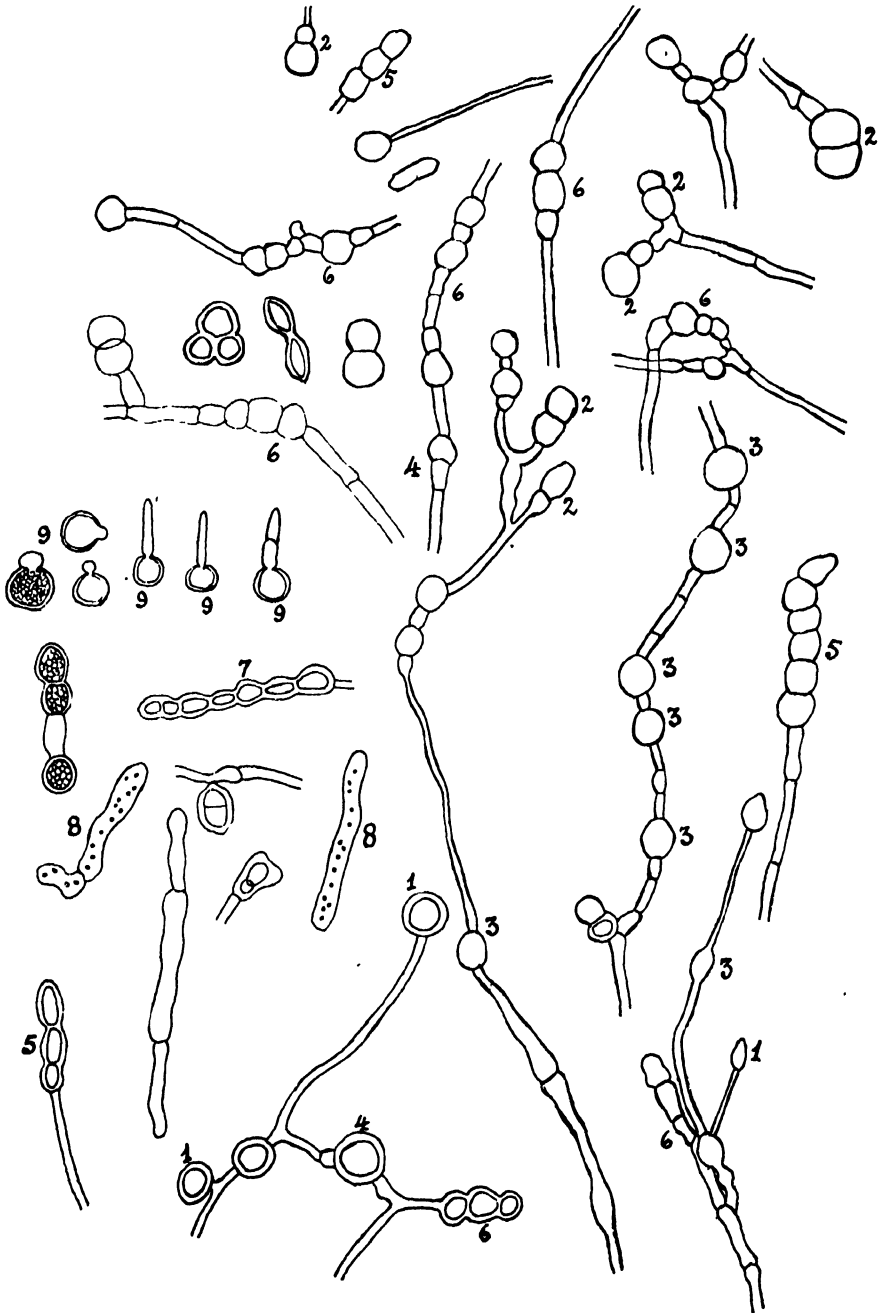


FIG. 8. Various stages of the American *Fusarium*.

CONTROL

The study of the control of the disease was divided into three parts: (a) the effect of the bare-fallow, (b) the disinfecting capacity of carbon bisulphide, (c) the selection of resistant long-staple cotton (Sakel).

Bare-fallow, and carbon bisulphide as soil injection, were found to be unsatisfactory as methods of control.

In 1923, 141 single plants were selected from heavily infected fields in Dakahlia and Menoufia provinces. These plants were from fields sown with Domains Sakel where infection was so severe that only a few plants were left standing by the end of the cotton season.

The progeny of the selected single plants were grown in heavily infected soil. Of these, plants of pure Sakel type which remained healthy were selected and sown again the following year in infected soil. The operations were repeated in 1925 and 1926 until finally four strains of resistant Sakel were obtained.

The lint of the mother-plants grown in 1925 and 1926 were graded as good Sakel by Mr. Charles Ross, of the British Egyptian Company, at Alexandria, and official grader of the Cotton Research Board of the Ministry of Agriculture.

The soil in which the selected cotton was grown was obtained from a heavily infected field and large quantities of parasitic cultures of the fungus were added. This soil was so heavily infected that 96 per cent of the seedlings of the ordinary Domains Sakel grown in it died from the disease.

To ensure complete exposure of the selected plants to infection, the infected soil was placed in bottomless pots buried in the field. The roots of the plants had in this way to grow through 50 centimeters of very heavily infected soil. Finally, in 1926, four strains of resistant Sakel cotton were obtained.

The seed of these four strains were enough to sow an acre and a half in 1927. Pure lines of these were grown in infected soil at Giza. The best plants of each strain will be selected and sown again the following year at Giza under the same conditions, while the bulk seed of each strain will be propagated at the experimental farm of Gemaiza or elsewhere. These operations will be repeated yearly, the nucleus plants being always grown at Giza in heavily infected soil, while fresh lots of bulk seed of each strain will be sown for propagation every year. In this way there will, eventually, be a continuous yearly flow of resistant Sakel seed from Giza through the propagation farms to the cultivators.

SUMMARY

1. The Fusarium wilt of cotton is so destructive to long-staple cotton in some localities of Lower Egypt that growers have been forced to sub-

stitute less valuable short-staple varieties which are resistant.

2. The prosperity of the country depends largely on the successful production of long-staple cotton; therefore it is essential to attempt to control the wilt.
3. The symptoms of the disease in Egypt and the morphology and some of the physiological characteristics of the causal organism are described.
4. In modified Richard's solution the pathogene produces a staling substance which causes cotton plants to wilt.
5. The optimum temperature for the growth of the fungus on acid potato plugs was 30°–35° C. The incubation period of the pathogene in the host varies with temperature, ranging from 58 days at 15.9° C. to 12 days at 26.9° C.
6. The pathogene seems able to penetrate cotton roots unaided by other soil organisms. It does not seem to be seed-borne in Egypt.
7. Heavy soil to which organic manure has been added is likely to be most heavily infected.
8. A comparative study was made of *Fusaria* causing cotton wilt in Egypt, India, and North America, respectively. There were some morphological and cultural differences. The three differ considerably in pathogenicity. The Egyptian form is virulent on long-staple Egyptian cotton, Sea-Island varieties, and can attack some of the Indian varieties; the Indian form is parasitic on some Indian varieties but not on American and Egyptian varieties; and the American form is non-parasitic on the Indian varieties tested, very weakly parasitic on Egyptian varieties, and parasitic on some American varieties.
9. Neither bare fallow nor the application of carbon bisulphide to the soil controlled the disease.
10. Four wilt-resistant strains of Sakel cotton were obtained by selection.

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A GLOEOSPORIUM BLIGHT OF RASPBERRY

B. O. DODGE

Orange-rust, cane blight, wilt or blue-stem, and spur blight are the best known fungous diseases of raspberries which are more or less systemic, and which result in the death of the affected laterals or main canes. For several years the writer has been observing some of the fungi which occur on different species, varieties, and new hybrids of *Rubus* in the breeding grounds of the Office of Horticulture at Bell, Maryland. One of the fungi most frequently found in early spring on the dead or dying canes of the previous season's growth is one which is probably the spur blight fungus. In certain morphological features, however—the presence of paraphyses in the ascocarp and the development of a pycnidial stage—the fungus does not altogether agree with previous descriptions of *Mycosphaerella rubina*. Spur blight is at first local in its attacks. The fungus may be found later throughout the length of the canes which it has killed. Sackett¹ has illustrated the early stages of this disease in his report. He has called attention to the gray patches that appear in the brown discolorations, and has noted that the black bodies seen on the gray patches are young ascocarps. He has not, perhaps, sufficiently described the appearance of the affected canes in the following spring, when ascocarps develop in great abundance over the entire surface, to the exclusion of most other fungi. In the spring the canes have a gray appearance. Such canes are likely to be very light and brittle and with but a limited amount of wood formed the previous season.

There is another disease which has also caused considerable damage to the red raspberry hybrids each year in the breeding plats at Bell, Md., and it is one which is being found in commercial plantings of black raspberries in Kentucky, Ohio, Michigan and other states. The writer is calling this disease Gloeosporium blight for reasons which will appear later. On the Cumberland variety it first causes a blackening or necrosis of the leaf stalks and tips of young shoots. Later the leaves collapse and the tip ends of the shoots turn purple or blue, the discoloration proceeding from the tip downward. The lower part of the young cane may remain green for some time. The whole turion may finally die or the disease may be confined to a single lateral. On account of the fact that the young canes, when badly infected, turn blue or purple the disease is likely to be confused during the summer season with the raspberry wilts which are known as blue-stem. In case of

¹ Sackett, W. G. Spur blight of the red raspberry caused by *Sphaerella rubina*. Colo. Agr. Exp. Sta. Bul. 206. 1915.

the raspberry wilts, the blue discoloration appears first at the base and works upward; while in the *Gloeosporium* blight the blueing of the shoot begins at the tips and works downward.

Spur blight first causes a brown discoloration of the affected parts. Later, after the perithecia have developed abundantly, the canes take on a gray appearance. It is in winter or early spring that the canes attacked during the previous summer by the *Gloeosporium* blight fungus present an appearance very similar to that of canes attacked by the spur blight organism. The entire cane may be infected (Plate XXVIII, A), or there may be only a broad, white or light gray streak of dead tissue along one side (Plate XXVIII, B); or there may merely be a dead area (Plate XXVIII, C) at the base of a dead lateral. Infected canes become girdled and eventually die without producing fruit, and in most cases without producing leaves.

A chromogenic *Gloeosporium* has been repeatedly isolated from the black oval spots on the gray canes each spring since 1922. Perithecia have been found in only one culture from all of this material. This culture, contaminated with bacteria, was obtained by transfer of a mass of conidia directly from an acervulus on a dead cane of the previous summer's growth. Pure cultures were made from this culture by the plating method. The new cultures happened to be all non-chromogenic. However, other sub-cultures from the original chromogenic culture on which there were ascocarps are invariably chromogenic. This seems to indicate that the original culture may have contained two different strains of *Gloeosporium*, one chromogenic and the other non-chromogenic. Ascocarps have developed in most of the new cultures of the non-chromogenic strain.² Shear and Wood³ report the appearance of *Glomerella cingulata* on diseased canes of blackberry received from Shelbyville, Tennessee, in March. Typical acervuli of the *Gloeosporium* stage developed on these canes soon after they had been placed in the damp chamber. Later many perithecia of *Glomerella* matured on this material. In cultures which were obtained from the discolored wood of the diseased canes and grown on cornmeal agar in tubes, mature perithecia were produced. These cultures were all non-chromogenic. The writer has obtained about 50 single ascospore cultures from the non-chromogenic strain from raspberries, which to date have remained non-chromogenic. All the other cultures made from the fungus on red raspberry hybrids at Bell, Maryland, and Rosslyn, Virginia, have been chromogenic.

² The perithecial stage has since been found on raspberries from several different states.

³ Shear, C. L. and A. K. Wood. Studies of fungous parasites belonging to the genus *Glomerella*. U. S. Dept. Agr. Bur. Plant Indus. Bul. 252. 1913.

Dr. Shear, who has examined not only the diseased raspberry material from Maryland, Virginia, Kentucky and Michigan, but also the various cultures obtained from these sources, informs the writer that he has been unable to find any morphological differences by which the raspberry strains can be distinguished from those commonly found on apples and referred to as *Glomerella cingulata* or *Gloeosporium cingulatum*. Shear and Wood have previously reported extensively on the great variability to be found in strains of this species as they occur on different hosts. They have made a large number of cross inoculations, which furnish evidence that the species frequently occurs on a wide range of hosts.

Infection of raspberry leaf stalks (Plate XXVIII, D) and shoots (Plate XXVIII, E) can readily be obtained either in the greenhouse or in the field by puncturing the cortex with a needle bearing conidia of the chromogenic strains. Within a very few days after making the inoculation, the cortex surrounding the puncture begins to shrink and turn brown or black. Within a week acervuli begin to develop (Plate XXVIII, E). New cultures from spores in acervuli on these artificially infected areas are chromogenic. When leaf stalks are punctured an inch or so from the base in making the inoculation, the mycelium spreads up and down fairly rapidly (Plate XXVIII, D), so that within a few days the leaf dies and falls. The fungus also may develop on leaves under certain conditions. When shoots were sprayed with spore suspensions, portions of several leaves turned brown and developed acervuli. These leaves may have been torn or scratched by thorns in the process of the experiments so that a way was opened for the invasion by the fungus. No evidence has yet been obtained that uninjured healthy raspberry shoots can be infected by merely spraying them with spore suspensions.

Only a limited amount of experimental work has been done with a view to determining whether bitter-rot strains of *Gloeosporium cingulatum* isolated from rotting apples will infect raspberries. Dr. Shear furnished the writer with a chromogenic and non-chromogenic strain from apple in order that this point might be tested out in a preliminary way. Leaf stalks and young growing canes of two different varieties of raspberry were inoculated with these strains by puncturing with a needle bearing conidia and then covering the wound with paraffin. Other canes were inoculated by forcing spore suspensions into young canes by means of a hypodermic needle. In some cases both of the strains from apples seemed to invade the living tissues of raspberry and form acervuli in about the same way as did the chromogenic strain from raspberry. One black raspberry shoot was punctured in nine different places. Infection followed in eight of the nine, and failure in the ninth may have been due to the fact that the wound had not been stopped with paraffin.

The raspberry and apple chromogenic strains developed at about the same rate and produced the same type of rot when inoculated into apples. The effects produced when apples and raspberries (Plate XXVIII, F) were inoculated with the non-chromogenic strain from apple were perhaps slightly different from those produced by the chromogenic strains referred to. There is no question in either case of the pathogenicity of the three strains on both hosts.

Material which Dr. W. D. Valleau has sent the writer from the Agricultural Experiment Station grounds at Lexington, Kentucky, indicates that certain varieties of black raspberry are very susceptible to infection by *Gloeosporium cingulatum*. On arrival the blackened tips of the young shoots (Pl. XXIX, A, b) showed masses of pink acervuli. Uncontaminated cultures were easily obtained by transfer of conidia directly from the acervuli on these blackened canes. When the canes were opened by splitting, one could see white mycelium penetrating the pith throughout the length of the blackened ends (Plate XXIX, B). The pith had turned brown or black and had become filled with pockets, due to drying out and cracking in sections. Many black raspberries were inoculated with the fungus from this material and all became infected.

In the *Gloeosporium* blight material from Kentucky and Michigan anthracnose (*Gloeosporium venetum*) could be seen in all stages of development, from the very earliest browning of small elevations surrounding the prickles to lesions with mature spores of anthracnose. Whether or not anthracnose very generally enters the young tissues of raspberry through hairs or through the primordia of prickles or thorns, there can be no question that most of the lesions in this material developed at the points of origin of prickles on the growing shoot tips. *Gloeosporium cingulatum* quickly established itself so that the anthracnose lesions contained acervuli of *G. cingulatum* as well as spores of *G. venetum*. Roberts⁴ has pointed out that the apple bitter-rot strain of *G. cingulatum* may overwinter by living in cankers and other lesions in apple trees caused by other fungi. As attempts to infect the raspberry by spraying uninjured canes with spore suspensions have been without positive results so far, it may be assumed that the fungus does not penetrate the unbroken epidermis under ordinary growing conditions. In one field in Michigan, where the *Gloeosporium* blight was fairly abundant, much of it was found on plants affected with yellow mosaic. Dr. C. W. Bennett, in calling the writer's attention to this association, suggested that a plant infected with this virus disease may in some way be predisposed to attack by this blight fungus.

⁴ Roberts, J. W. Sources of the early infection of apple bitter-rot. Jour. Agr. Res. 4: 59-64. 1915.

Just how much anthracnose, *Gloeosporium venetum*, may ordinarily be a factor contributing to the original infection of the raspberry canes by *G. cingulatum* can not be stated definitely here. All of the material sent by Valteau had anthracnose lesions on the green shoots and laterals (Plate XXIX, A, a). In most cases, as noted previously, these lesions contained acervuli of *G. cingulatum* in addition to the spores of the anthracnose organism. After photographing this material, the branch "a" (Plate XXIX, A) was cut off and the end placed in water. At that time the branch was turgid and green except where spotted with the anthracnose. Two days later the entire tip had become blighted and purplish black, and acervuli were breaking out through the cortex over the entire length of the infected portion. When this branch was split open it was found that the pith had also turned brown and had become pocketed to within two inches of the base (Plate XXIX, C). Evidently the blight fungus had spread very rapidly from each of the anthracnose lesions.

While *Gloeosporium* blight of raspberries in Maryland and Virginia has not been so serious as to interfere greatly with the breeding experiments in progress, evidence furnished by Valteau from Kentucky shows that under certain conditions this blight may become an important factor in limiting production in southern regions. Should further work demonstrate beyond a doubt that the anthracnose lesions referred to on raspberry canes contribute to the spread of the *Gloeosporium* blight, methods of controlling the blight would involve more effective control of anthracnose by spraying or dusting.

SUMMARY

Gloeosporium blight is reported on red raspberry hybrids in Maryland and Virginia and on commercial varieties of black raspberries in Kentucky, Michigan, Ohio and other states.

The symptoms of the disease are described and differentiated from those of spur blight and blue stem or wilt.

A chromogenic *Gloeosporium* has been isolated from blighted red raspberry canes at Bell, Md., each spring since 1922.

Pure cultures of a non-chromogenic strain of the fungus were obtained by the plating method from a chromogenic culture which was contaminated with bacteria.

According to Shear, the strains of *Gloeosporium* isolated from raspberries cannot be distinguished morphologically from those commonly found on apples and referred to as *Glomerella cingulata* or *Gloeosporium cingulatum*.

Cross inoculations with the chromogenic strain from raspberry and with a chromogenic and a non-chromogenic strain from apple demonstrated the pathogenicity of the three strains on both hosts.

Attempts to infect the raspberry by spraying uninjured canes with spore suspensions indicate that the fungus does not readily penetrate the unbroken epidermis under ordinary growing conditions.

Preliminary studies suggest that anthracnose lesions may open the way for invasion by *G. cingulatum*.

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EXPLANATION OF PLATES

PLATE XXVIII

Gloeosporium blight of raspberry.

A. Portion of cane of Van Fleet raspberry in April, showing effects of natural infection of the growing shoot the previous summer by *Gloeosporium cingulatum*. Black acervuli scattered evenly over the entire surface.

B. Whitish dead streak of tissue on one side of a cane of a red raspberry hybrid at Bell, Maryland, in April. Acervuli were abundant on the dead portion of this cane.

C. Infected main cane. The fungus must have passed down into the main cane after having killed the lateral, a.

D. Leaf stalk of a hybrid red raspberry in the greenhouse ten days after inoculation with a chromogenic strain of *Gloeosporium cingulatum* from apple.

E. Main stem of a young shoot of the plant shown in E. Acervuli developing on the lesion.

F. Black raspberry shoot two weeks after inoculation with the non-chromogenic strain of *Gloeosporium cingulatum* from apple.

A, D, E, and F enlarged.

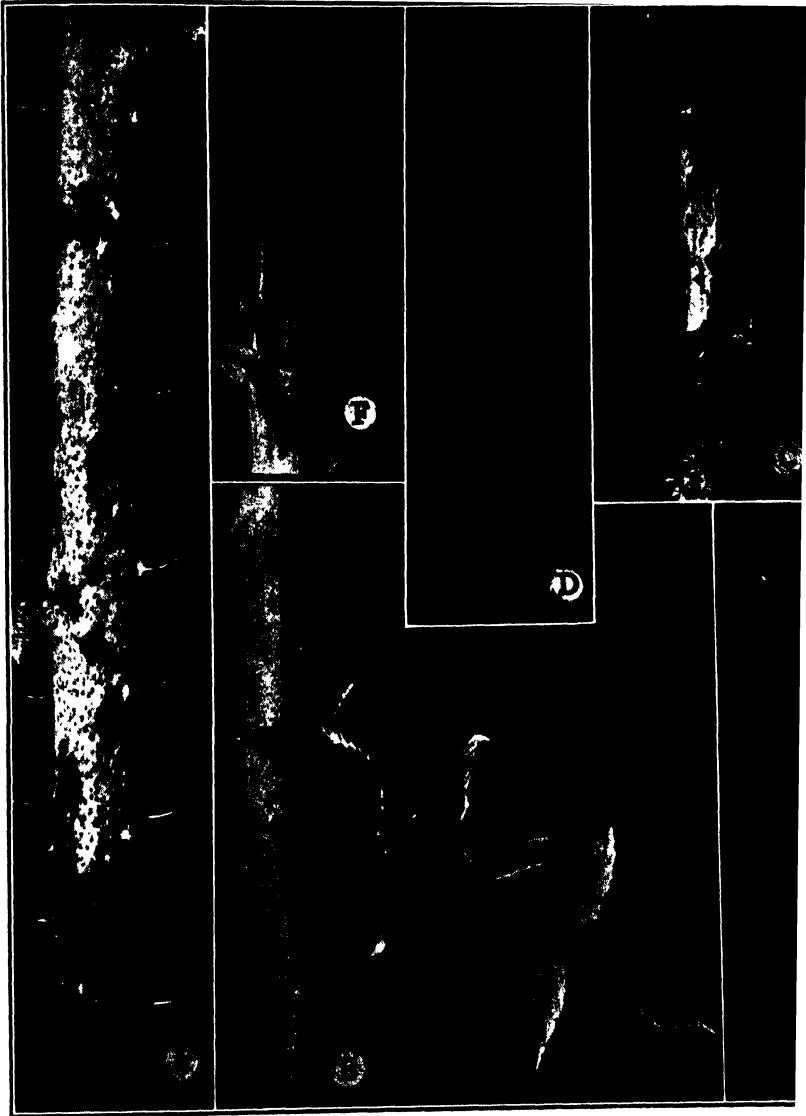
PLATE XXIX

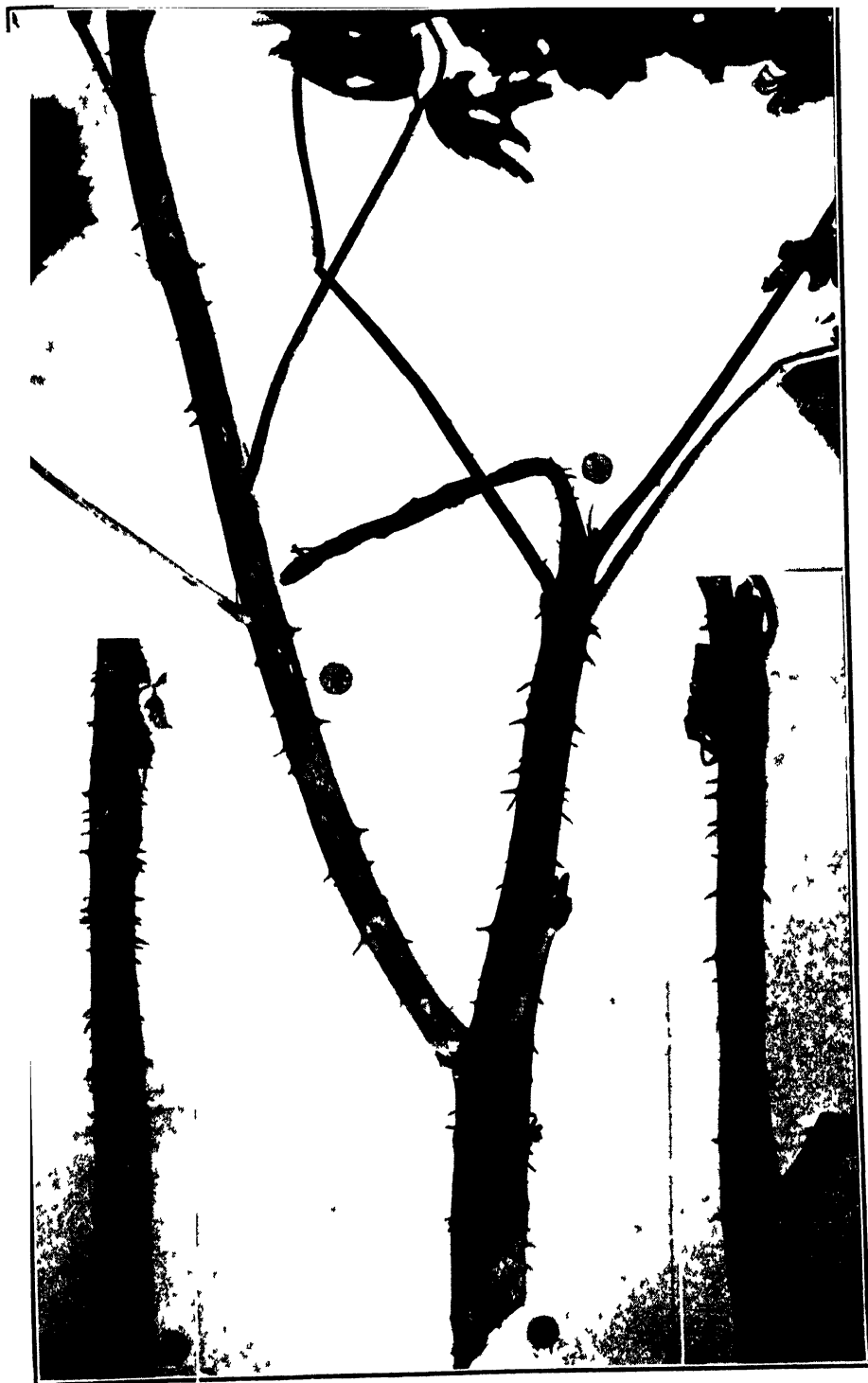
Gloeosporium blight on cultivated black raspberry received from Dr. W. D. Valleau, Lexington, Kentucky.

A. The blighted tip end (b) and leaves of the main cane. The bluish-purple dead end was covered with acervuli of *G. cingulatum*; at the left, a green branch (a) spotted with anthracnose lesions, each of which also contained acervuli of *G. cingulatum*.

B. Tip end of a blighted young cane split open to show the brown pith with numerous cavities in which a white mycelium of *G. cingulatum* was maturing numerous conidia.

C. Part of the branch (a) shown in A, after it had been cut off and the end placed in water for nearly three days. The upper part had collapsed and turned bluish-black. Large numbers of acervuli were beginning to break through the epidermis. Numerous cavities had been formed in the pith. At the lower end, which was still green, the pith was solid.





A POTATO VIRUS ON PEPPERS

F. M. BLODGETT

Early in 1925 attempts were made to inoculate pepper plants with the virus of yellow dwarf disease of potatoes. Fortunately, at the same time, inoculations were made on peppers with inoculum from apparently healthy potato tubers and from mosaic and healthy tobacco. On all of the peppers inoculated (February 16) from potato, both from the yellow dwarf tubers and from the healthy tubers, striking symptoms developed (March 2) quite different from those which developed on the plants inoculated with tobacco mosaic virus. The peppers inoculated with inoculum from healthy tobacco remained healthy.

This result was immediately recognized as similar to the then unpublished results of Fernow (1) with inoculations from apparently healthy potatoes to *Nicandra physalodes* (L.) Pers. and to *Nicotiana glutinosa* L., and similar to the results of Johnson (2, 3) with inoculations from potatoes to tobacco. Further work with this disease on pepper has confirmed in many respects the work of the above-mentioned experimenters. In the meantime, in his bulletin, Johnson (2) has listed pepper among the plants susceptible to one or more of the virus diseases which he obtained from potatoes. In a more recent article Schultz (4) has reported finding certain potatoes susceptible to a virus from other apparently healthy potatoes.

The symptoms produced on pepper with the virus from apparently healthy potatoes were so striking that this host was believed to be a favorable one on which to test the theory then just suggested by Johnson in his abstract at the Washington meeting of the American Phytopathological Society, namely, that something in the extract of normal potato foliage causes a disease of tobacco and tomato which is of an infectious nature. Some of Johnson's experimental plants had been seen by the writer, and the results could not be doubted. It was not found easy to duplicate this work, however. Difficulty was experienced in growing tobacco and tomatoes under conditions sufficiently favorable so that the symptoms could be seen, and at best they were not very striking. It seemed particularly pertinent to find out whether the same virus could be obtained from seedling potatoes before these had been infected from commercial potatoes. In other words, if the virus is to be considered as some normal constituent of potato foliage, a necessary condition would seem to be that it should be found in the foliage of seedling potatoes.

In the experiments to be described here, inoculations were made with a flamed needle on which a tuft of absorbent cotton was wound to act as a holder of inoculum. The material to serve as inoculum was ground in a steam sterilized mortar. A fairly regular, although entirely arbitrary, number of punctures was made in each plant: ten in the stem and ten in each of three leaves.

The symptoms produced on peppers by inoculations with the virus from apparently healthy potatoes varied considerably in time of appearance, as in some other mosaics, depending on conditions of growth and temperature. In parallel inoculations they were a few days slower in appearing on peppers inoculated with the virus from potatoes than on those inoculated with tobacco mosaic virus. Under favorable conditions they appeared in ten days.

The first symptoms to be observed are rather indefinite, light spots in



FIG. 1. Pepper plant inoculated from an apparently healthy potato. Necrotic spots have developed in the leaves, and the leaves are more or less distorted.

the young leaves developed after inoculation. These spots quickly die and turn brown. They are variable in shape, and in size up to about a half centimeter in diameter. As the spots are developed in the rapidly expanding younger leaves, these leaves frequently become wrinkled and distorted (Fig. 1). They do not persist on the plants for long, but drop within a few days leaving the plant with only the older leaves developed previous to the virus infection (Fig. 2). Necrotic streaks spreading from points of inoculation are developed frequently also in the stems (Fig. 2). For comparison, a healthy plant is shown in figure 3.

Plants affected as described, however, usually do not die completely. In a short time, after one crop of new leaves has been shed, more buds begin to push out if the plant is kept under conditions favorable for growth. The leaves appear quite normal until about half grown. They then develop lighter colored areas which frequently give them a decidedly mosaic appearance. Soon the light areas become necrotic, the leaves drop, the plant remains dormant for a brief period, and then starts anew the succession of symptoms as described. Such plants naturally remain very much dwarfed. One such plant, however, has remained alive in the greenhouse for over two years.

TABLE 1.—*Results of inoculating tobacco and pepper plants with a virus from different varieties of potato, July 23 to August 4, 1925*

Variety of potatoes used as source of inoculum	Pepper plants		Tobacco plants	
	No. inoculated	No. infected	No. inoculated	No. infected
Green Mountains	4	4	4	4
No. 9	4	4	4	4
Seedlings	4	0	4	0
Cobblers	4	3	4	4
Rural Russets	4	4	4	4
Heavy Weight	4	3	4	4
Bliss Triumph	4	2	4	2
None (check)	5	0	31	0

In this way peppers have become infected in various tests extending over a period of a year and a half. In all, twenty-five lots of pepper plants were inoculated at different times. Of 135 plants inoculated with the virus from various commercial varieties of potatoes, 114 plants became infected and symptoms were as described above. Both punctured and unpunctured checks, 105 in number, have been grown with inoculated plants but have regularly remained free from this disease. In fact, there has been no indication that the disease spreads in the greenhouse.

No extensive search has been made to discover a plant of a commercial variety of potatoes free from this virus, although a few inoculations have

been made from individual potato plants. In March, 1925, fifteen pepper plants were inoculated, each from a different potato plant. Eleven of the fifteen became infected. In a parallel experiment *Nicandra* was inoculated from the same potato plants, and infection was secured from two additional potato plants. In a later experiment, virus from eight Bliss Triumph potato plants was used to inoculate eight sets of five pepper plants each. In these cases the virus from each of the potato plants caused infection in each set of five pepper plants except in one case in which only three of the five became diseased. All the different commercial varieties of potatoes used appear to be carriers of this virus. The results of one experiment in which peppers and tobacco were inoculated with the virus from different varieties of potatoes are given in table 1. It will be noted that all of the varieties except the seedlings proved to be carriers of this virus. The symptoms obtained on tobacco seemed to be of Johnson's "mottle" type and this has been the type regularly obtained from the virus of the potatoes used in these tests where parallel inoculations have been made on tobacco. No differences were noted in the symptoms on peppers inoculated with the virus from these different varieties of potatoes.



FIG. 2. A little later stage than is shown in figure 1. Most of the affected younger leaves have dropped. The stem shows a necrotic streak

As noted in one case in table 1, in a number of instances the inoculum was taken from seedling potatoes, *i.e.*, plants grown from true seed. Most of these seedlings were grown from seed produced by the variety Green Mountain. In all, 57 pepper plants were inoculated in 12 different lots with inoculum from seedling potatoes, but in none of these cases did any symptoms develop on the peppers to indicate that a virus had been transmitted.

Seedling potatoes were inoculated with the virus from commercial potatoes at various times, but no symptoms of disease were observed on the seedlings. Three of these were the same ones previously used as a source of inoculum for peppers. The results of inoculating peppers also were negative. Later, at several different times, peppers were inoculated with the virus from these inoculated seedling potatoes. Of 33 pepper plants inoculated, 14 became infected. Equal numbers of pepper plants were inoculated from uninoculated seedling potatoes of the same age and lots in each case but did not become infected. Thus it appears that seedling potatoes, until inoculated, are free from the virus which commonly is carried by the commercial varieties of potato. The percentage of infection on the peppers inoculated from the inoculated seedlings was low, as might be expected in using inoculum from plants with no visible symptoms to serve as guide as to which of the previous inoculations had been successful. The symptoms of this disease on peppers are so striking that no doubt can be entertained in the case of positive infection.

After this potato virus has been transferred to pepper, it may be readily transferred from one pepper to another. When the virus from one of the first peppers inoculated from potatoes was used to inoculate a second lot of 10 peppers, 9 became diseased. A second transfer from one of these peppers to 5 more resulted in all 5 becoming diseased. No difference was noted in the symptoms of the disease on these different lots.

An attempt was made to see whether this potato virus disease is seed-transmitted in peppers. When pepper fruits produced on diseased plants were examined, it was found that they contained very few seed as compared with the fruits on healthy plants. A few seed were obtained, however. They were gathered October 12 and planted November 7, 1925. The seedlings (not counted) raised from these seed all appeared healthy. In addition, 21 of these transplanted to pots continued to look healthy until five of them were inoculated on January 18. Four of these became infected. Although this test was not very extensive, it indicates that the potato virus is not transmitted through pepper seed at least to any considerable extent, and that plants raised from seed produced on diseased plants are still susceptible to the potato virus disease.

No symptoms were detected when Bliss Triumph potatoes were inoculated with the potato virus from diseased peppers. Fifty potato plants were inoculated in April, 1925, with the potato virus from diseased peppers. These were kept until they died in July. Parallel inoculations were made with material from healthy peppers, the potato virus (Johnson's "mottle") from tobacco, and mild mosaic of potatoes. A large number of uninoculated plants was kept for comparison. No symptoms of disease were observed on any of these plants. When some of the progeny were grown, the only disease which developed was mild mosaic. Results were as follows: 3 plants with mild mosaic of 39 inoculated with potato virus from pepper, 0 out of



FIG. 3. A healthy pepper plant of the same age as those shown in figures 1 and 2.

27 inoculated with potato virus from tobacco, 10 out of 18 inoculated with mild mosaic, and 2 of 34 uninoculated. While the results of this series of inoculations are unsatisfactory, showing as they do only low percentages of infection when inoculations were made with the virus of mild mosaic and probably a natural spread of mild mosaic to some extent in the whole series, certainly no striking necrotic symptoms were secured. So far as this evidence goes, it indicates that the disease in question in this work was not Johnson's "spot-necrosis," but it does correspond with his "mottle."

So far, only a few plants having this disease have been seen in commercial fields, but only a few fields of peppers have been examined since these experiments were begun. How important the disease is commercially is not known. It might naturally be expected to occur in peppers when planted near potatoes, especially if an insect carrier is present.

SUMMARY

A serious disease of peppers is caused by inoculating them with the virus from apparently healthy commercial varieties of potatoes. This is apparently caused by the same virus mosaic B from potatoes that Fernow has used in inoculating *Nicandra physalodes* and *Nicotiana glutinosa* and some six other hosts, and which Johnson has described as the cause of "mottle" on tobacco and which he has shown may be transmitted to some thirty species of plants.

The symptoms of the "mottle" potato mosaic on pepper are marked. Faint mottling develops on leaves about half grown. The light colored areas quickly become necrotic, and affected leaves drop shortly. Necrotic areas spreading from inoculation points frequently develop on the stems. The plants are dwarfed. The only potatoes found from which the potato "mottle" virus could not be obtained were seedling potatoes from true seed. The virus could be transmitted to such seedlings by inoculation.

The potato virus is transmitted readily from one pepper to another by inoculation.

The virus is neither transmitted through pepper seed nor potato seed, although it is transmitted through potato tubers.

The extent of the occurrence of this disease in commercial fields is not known.

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PHYSIOLOGIC SPECIALIZATION IN PUCCINIA CORONATA AVENAE

H. E. PARSON¹

INTRODUCTION

In 1919 Hoerner (3) demonstrated that there are physiologic forms of *Puccinia coronata avenae* (Corda) Erikss. and Henn. which differ in their pathogenicity on certain varieties of oats. There has been but little, if any, subsequent investigation of the problem. In fact, Hoerner's results never have been amplified, or even confirmed. And, as far as the writer knows, no attempt has been made to take into consideration the established fact of physiologic specialization in developing varieties of oats resistant to crown rust.

It is quite likely that crown rust causes greater aggregate losses to oats in the United States than does stem rust. In some of the southern oats-growing sections crown rust is very destructive while stem rust is relatively unimportant. In the upper Mississippi Valley crown rust often causes heavy losses in the vicinity of buckthorn bushes; and sometimes, as in 1927, general epidemics develop and reduce yields greatly.

Something should be done to reduce losses from crown rust. In many of the northern oats-growing states the eradication of buckthorn bushes would reduce losses considerably, but this would not be true of the Southern States. Even if the eradication of the buckthorn would reduce losses greatly in the Northern States, it is doubtful whether it would be feasible to organize and successfully complete an extensive eradication campaign. And the writer is convinced from personal observation that the buckthorns will remain and multiply forever unless such a campaign is begun soon. Crown rust will become increasingly destructive in the Northern States as the number of buckthorn bushes increases, and the rust will continue to take its annual toll in the Southern States—unless resistant varieties of oats are produced.

The development of varieties of oats resistant both to crown rust and stem rust may not be easy. It would be most desirable to breed varieties

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resistant to the two rusts, the smuts, and spikelet sterility. Anthony (Minn. 686) derived from White Tartar \times Victory has been resistant to stem rust in many widely separated localities in the United States, but it is very susceptible to crown rust and to the smuts. Markton (C. I. 2053), on the other hand, is very resistant to the two smuts, but susceptible to the rusts. The whole problem is complex because there are physiologic forms of *Ustilago avenae* and *U. levis* (5), of *Puccinia graminis avenae* (1, 9) and *P. coronata avenae*. However, as the smuts can be controlled by seed treatment, it would be a valuable contribution to develop varieties resistant to the two rusts. If this contribution is to be made, the physiologic specialization of the rusts must be investigated and the facts obtained must be used in breeding work. For this reason the present study was made.

MATERIALS AND METHODS

One way of determining whether there are physiologic forms of a pathogenic fungus is to inoculate varieties ordinarily resistant with collections from different places. If the varieties are susceptible to any of the collections, it is evidence of the existence of forms. On the other hand, if any usually susceptible varieties are resistant, there also would be evidence of the existence of forms. In the present work the writer selected a number of varieties of oats which had been reported as resistant to crown rust (2, 4, 6). These were inoculated with collections of rust obtained from fifteen different places in the United States and Canada.²

Most of these collections were from cultivated oats, but a few were obtained from *Rhamnus* spp. The rusted material was sent to University Farm, St. Paul, and inoculations were made as soon after arrival as possible.

The inoculation technique was similar to that described by Stakman and Piemeisel (7). Victory oats was used for stock cultures, although Richland was used in a few cases.

RESULTS

¹ It was quite evident that there were distinct differences in the pathogenicity of the different collections of rust obtained. The types of infection described by Stakman and Levine (8) for *P. graminis tritici* all appeared with the exception of type 2. Type 3 sometimes approached type 2, but no clear-cut type 2 was ever observed. For convenience, the descriptions of these types are given below.

² The writer wishes to acknowledge his indebtedness to the pathologists and agronomists who were kind enough to send rust and seed material, and to T. R. Stanton, Office of Cereal Crops and Diseases, United States Department of Agriculture, for seed of some of the varieties of oats.

“0—*Immune*

“No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues

“1—*Very Resistant*

“Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas

“2—*Moderately Resistant*

“Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands

“3—*Moderately Susceptible*

“Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present

“4—*Very Susceptible*

“Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.”

Plants were considered resistant if they developed a type 1 infection. If they developed a type 3 or type 4, they were considered susceptible. In the determination of forms, resistance and susceptibility only were taken into consideration.

The results of the inoculations are summarized in table 1. It shows clearly that the different collections naturally fall into several groups with respect to their pathogenicity. All of the varieties inoculated are susceptible to the collections in group 1. The rusts in this group, therefore, are considered to be form 1. In the second group all varieties are susceptible, except Green Mountain, which is resistant. The difference between collections in this group and those in the first group is simply in their effect on Green Mountain. The collections in this second group are considered to be form 2. *Avena sterilis nigra*, *A. sterilis* selection, Burt, Red Rustproof, and Ruakura are all resistant to form 3, while Green Mountain is susceptible to this form. Ruakura is the only variety resistant to form 4. The four forms are very distinct. In fact, the differences in their pathogenicity are so sharp that one is forced to conclude that they are distinct physiologic forms. The differences are quite as pronounced as are the differences between physiologic forms of *P. graminis tritici*. There is a possibility that there is a fifth form. The collection obtained from Ste. Anne de la Pocatiere, Quebec, is quite similar to form 3, except for the fact that *A. sterilis nigra* and *A. sterilis* selection are moderately susceptible to the Ste. Anne collection instead of being resistant. There is a wide difference in the reaction of these varieties to the two rusts, and it seems justifiable, therefore,

TABLE 1.—Reaction of 27 species, varieties, and selections of *Avena* to *Puccinia coronata avenae* collected in various places in the United States and Canada in 1926 and 1927

Varieties of oats inoculated	Place of collection and rust reaction														
	Group 1								Group 2			Group 3		Group 4	Group 5
	A and M. College, Miss.	Chatham, Mich.	Columbia, Mo.	Denton, Tex. (Collection No. 20)	San Antonio, Tex.	Stillwater, Okla.	Tifton, Ga.	Yankton, S. Dak.	Durant, Okla.	Lincoln, Nebr.	Mill Creek, Okla.	Omaha, Nebr.	St. Paul, Minn.	Denton, Tex. (Collection No. 11)	Sta. Anne de la Pocatieri, Que., Can.
Green Mountain.....	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S
<i>A. sterilis nigra</i> ^a	S	S	S	S	S	S	S	S	S	S	S	R	R	S	MS ^b
<i>A. sterilis</i> selection ^a	S	S	S	S	S	S	S	S	S	S	S	R	R	S	MS ^b
Red Rustproof.....	S	S	S	-	S	-	S	-	S	S	S	R	R	S	R
Red Rustproof, C. I. 1039.....	S	S	S	-	S	-	S	-	S	S	S	R	R	S	R
Red Rustproof, C. I. 1815.....	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
Ruakura, C. I. 2025.....	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R
Burt.....	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
Burt, C. I. 2042.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Burt 916, C. I. 2054.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
<i>Avena barbata</i>	-	-	-	-	S	-	-	-	-	S	S	-	S	-	-
<i>A. brevis</i> , colored.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
<i>A. brevis</i> , white.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
<i>A. nuda</i>	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
<i>A. sterilis ludoviciana</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
<i>A. strigosa</i>	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
C. I. 606.....	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Culberson.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Culred, C. I. 518-189.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Fulghum, C. I. 708.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Green Russian.....	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Kanota.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Kanota, C. I. 839.....	-	-	-	-	S	-	-	-	S	-	-	-	S	-	-
King.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
Rustless, C. I. 724.....	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S
White Russian, C. I. 1871.....	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Winter Turf ^c	S	S	S	-	S	-	S	-	S	S	S	S	S	S	S

^a There was some evidence on the basis of the reaction to the rust that these were not pure lines.

^b The uredinia were fairly large but were surrounded by pronounced necrotic areas.

^c The varieties here listed without numbers are those of Etheridge, obtained through the Agronomy Department of the University of Minnesota.

to conclude, at least tentatively, that the Ste. Anne collection represents a fifth form.

The differences in the infection ability of these five forms are shown more clearly in table 2. In this table are listed only the four varieties which served as differential hosts.

TABLE 2.—Reaction of four differential varieties of oats to five physiologic forms of *Puccinia coronata avenae*

Variety	Physiologic form and place of collection*				
	1	2	3	4	5
	San Antonio, Tex.	Durant, Okla.	St. Paul, Minn.	Denton, Tex.	Ste. Anne de la Pocatiere, Que., Canada
<i>Avena sterilis nigra</i>	S	S	R	S	MS
Red Rustproof, C. I 1815.....	S	S	R	S	R
Ruakura, C. I. 2025.....	S	S	R	R	R
Green Mountain	S	R	S	S	S

* The writer is indebted to the following for the collections of rust: to Wallace Butler for Nos. 1 and 2; to P. B. Dunkle for No. 4; to H. N. Racicot for No. 5. No. 3 was obtained from stock cultures growing in the Plant Pathology greenhouses at University Farm, St. Paul.

The writer has made an analytical key for the identification of the five physiologic forms of *Puccinia coronata avenae* within the genus *Avena*:

- Ruakura—resistant (Hoerner’s Form 3)
 - Avena sterilis nigra*—resistant Form 3
 - A. sterilis nigra*—susceptible
 - Red Rustproof—resistant Form 5
 - Red Rustproof—susceptible Form 4
- Ruakura—susceptible (Hoerner’s Form 1)
 - Green Mountain—resistant Form 2
 - Green Mountain—susceptible Form 1

The question naturally arises as to whether some of the forms which the writer isolated were identical with those which Hoerner described. Hoerner used Ruakura and Green Russian as differential hosts. He distinguished four forms as follows:

- Form 1. Infects both Iowa 73 [Ruakura] and Iowa 96 [Green Russian] normally.
- Form 2. Infects both varieties weakly.

Form 3. Infects Iowa 73 weakly but infects Iowa 96 normally.

Form 4. Infects Iowa 73 normally but infects Iowa 96 weakly.

As Green Russian was susceptible to all of the collections of rust which the writer tried, it is evident that Hoerner's forms 2 and 4 were not found in the present study, unless the Green Russian material used by the writer was different from that used by Hoerner. There is a possibility that Hoerner's form 1 could be either identical with the writer's form 1 or 2, or might be separated into forms 1 and 2 described in this paper. Furthermore, if the collection from Ste. Anne is a fifth form, as it appears to be, it would be possible for Hoerner's form 3 to be either form 3, 4, or 5 described in this paper, or consist of a combination of any two of these forms, or all three of them (see key).

It is entirely possible, of course, that the forms described in this paper could be sub-divided still further if more varieties of oats were inoculated. It may be possible also that other differential hosts would prove better than those which the writer used. It is likely that there are a great many physiologic forms, and the chance of detecting them simply depends upon making many inoculations on many different varieties of oats, and possibly on wild grasses.

Some of the physiologic forms differ from each other in the readiness with which they produce telia. Some of the forms never produced telia during the entire course of the experiment, while others consistently produced them about three weeks after inoculation. Parker (4) was of the opinion that early production of the telia was indicative of resistance of the host plants. However, in the writer's experiments, telia were formed by certain forms as early on susceptible varieties as on resistant ones. All of the writer's experience indicates that the early production of telia is due to an inherent tendency of a particular physiologic form of rust rather than to resistance of the host plants. Form 3 showed a marked tendency to produce telia. This form infects fewer varieties than any of the other forms. It is possible that the early formation of telia is characteristic of narrowly specialized forms. However, further investigations are needed before this conclusion can be drawn finally.

CONCLUSIONS

There can be no question that there are physiologic forms of *P. coronata avenae*. Hoerner was able to recognize four on the basis of their effect on only two varieties of oats. The writer has been able to recognize five by their effect on four differential hosts of *Avena* spp. The types of infection produced by these different forms are distinct and constant. It is rather remarkable that all of the varieties inoculated are susceptible to form 1, and

all but one (Green Mountain) are susceptible to form 2. All of the differential hosts are resistant to form 3 except Green Mountain, which is susceptible. These differences have been consistent after repeated inoculations. There can be no question but that these physiologic forms differ from each other genotypically. Further proof of differences is the fact that some forms have a tendency to produce telia earlier than others. This tendency is quite independent of the resistance or susceptibility of the host but seems to be a genetic characteristic of the physiologic form of rust. It seems likely that there is a correlation between the narrow pathogenic specialization of a strain and early formation of telia, but this cannot yet be stated with certainty.

Crown rust often causes heavy losses both in the Southern States and in the Northern States. In the South the urediniospores apparently survive the winter and the rust is independent of the alternate host, the buckthorn. In the Northern States, however, there seems to be abundant evidence that destructive epidemics of crown rust usually occur only near buckthorn bushes. However, in 1927 a widespread and destructive epidemic developed. It is doubtful whether this could have been traceable wholly to buckthorns. The only solution of the crown rust problem in the South would seem to be the development of resistant varieties. While the eradication of the buckthorns from the Northern States undoubtedly would reduce losses in most years, it is doubtful whether the eradication of the bushes could be successfully accomplished. Therefore the development of resistant varieties for the Northern States also is desirable. In order that such breeding work may be placed on a sound basis, it would seem highly desirable to make an extensive study of the number, distribution, and pathogenic capabilities of the physiologic forms of *P. coronata avenae*.

SUMMARY

1. The writer obtained collections of *P. coronata avenae* from fifteen different places in the United States and Canada. Twenty-seven varieties, selections, and species of cultivated and wild oats were inoculated with these collections.

2. Four varieties of oats proved to be differential hosts. On the basis of the reaction of these varieties to the different collections of rust, it was possible to distinguish four, and possibly five, physiologic forms. It is very likely that many other forms can be recognized if more extensive work is done.

3. Physiologic forms of *P. coronata avenae* differ in their effect on host plants. In some cases the effect on a single host determines the identity of the form.

4. The physiologic forms apparently differ also in the readiness with which they produce telia. The production of telia seems to be independent of the resistance or susceptibility of the variety on which the rust is growing. It seems rather to be due to an inherent tendency within the forms.

5. In developing varieties of oats resistant to crown rust, it will be necessary to take into consideration the physiologic specialization of the pathogene.

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PHYTOPATHOLOGICAL NOTES

Two Fungi on Sclerotinia Apothecia.—In the course of experiments with apothecia of the peach brown-rot fungus, *Sclerotinia americana* (*S. fructicola*), two other fungi were at times prominent as disturbing elements. They were seen first, in the spring of 1921, on apothecia developing from brown-rot mummies which were collected in a local peach orchard and kept in the greenhouse at 10–22° C. When first noted, about a third of the apothecia present were coated with a dense white mycelial growth. This spread rapidly, attacking small developing apothecia as well as the larger mature ones. The apothecia in one box were not infected and were still actively sporulating after the diseased apothecia had dried up. Upon microscopic examination two fungi were found, the most common of which was a *Fusarium* and the other a *Trichoderma* (*T. lignorum* or *koningi*).¹

In the spring of 1922 inoculations were made with cultures saved from the previous year and *Sclerotinia* apothecia grown from mummies gathered in the orchard after the developing apothecia were already visible. The apothecia were allowed to develop thereafter in the laboratory, using the method employed for physiological experiments with apothecia in which peach mummies were suspended by chromel wire in tumblers half filled with distilled water, the bottoms of the mummies dipping into the water.² To keep the atmosphere saturated, each tumbler was covered with a dish. In the present experiment the room temperature varied daily from about 15° to 30° C. Under these conditions *Sclerotinia* apothecia grow readily and apparently quite naturally.

Two peach mummies, each bearing apothecia ranging from mature ones to those that had not yet formed disks, were used for each of the organisms. A bit of material from the culture was deposited lightly on one apothecium per mummy in each case. Both *Fusarium* inoculations were plainly successful in two days, a heavy, white growth of *Fusarium* hyphae showing on the inoculated apothecia. In two more days the fungus had spread to other apothecia (Fig. 1), and three days later every apothecium on the inoculated mummies was obscured in a mass of mycelium. The *Fusarium* was re-isolated readily from these diseased apothecia. No infection occurred on apothecia inoculated with the *Trichoderma* or on the checks.

¹ Cultures of these organisms were lost by mite infestation before more definite identification was attempted.

² Norton, J. B. S., W. N. Ezekiel, and R. A. Jehle. Fruit-rotting *Sclerotinias* I. Apothecia of the brown-rot fungus. Md. Agr. Exp. Sta. Bul. 256: 3–32. 1923. (See figs. 17 and 18.)



FIG. 1. *Fusarium* sp. on apothecia of *Sclerotinia americana*. Photographed four days after apothecium (A) was inoculated. Note hyphal spread as far as (B). $\times 1 \frac{1}{3}$.

Both organisms were frequently found in the spring of 1922 on apothecia growing under experimental conditions such as those described above. The *Trichoderma* was observed chiefly on apothecia injured by the various toxic substrata to which they had been exposed; while the *Fusarium* was found on vigorously growing apothecia also, in agreement with the inoculation results.

Macroscopically the two fungi may appear rather similar on apothecia until spores are produced, when the dark green, mound-like masses of the *Trichoderma* are readily distinguished from the pinkish *Fusarium*.

The writer has never observed this fungus injury to apothecia in the field. Even though these organisms seem not uncommon on peach brown-rot mummies, it is unlikely that even the *Fusarium* is of any considerable importance in the destruction of apothecia under natural conditions. Fortunately, the discovery or dissemination of such natural enemies of this *Sclerotinia* has little practical significance, as the dangerous source of brown-rot infection from apothecia can be controlled otherwise, for instance by the old practice of burying mummies by plowing or other cultivation, which not only prevents apothecial development but also hastens disintegration of the mummies themselves.³—WALTER N. EZEKIEL, Maryland Agricultural Experiment Station, College Park, Md.

³ Ezekiel, Walter N. Fruit-rotting *Sclerotinias* III. Longevity of buried brown-rot mummies. Md. Agr. Exp. Sta. Bul. 284: 9-22. 1926.

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SEED TREATMENT CONTROL OF RHIZOCTONIA OF POTATOES IN IDAHO¹

J. M. RAEDER AND C. W. HUNGERFORD

INTRODUCTION

Experiments conducted in 1924 and in previous years² with various potato seed treatments were continued in 1925 and 1926 at the Idaho Agricultural Experiment Station. These tests, although not identical in every respect for the three years, were nevertheless similar enough, in methods of application, procedure and tabulation, so that comparison of the various years' results can readily be made.

The reason these treatments were not identical was that, from year to year, certain methods of treatment or certain compounds were found unsatisfactory and discontinued, or new compounds appeared which were worthy of trial.

Considerable emphasis was placed upon presprinkling. By this is meant the thorough wetting of each tuber to be treated 48 hours previous to treatment, then piling and covering, in order to keep them moist during that time. The same procedure was followed in 1925 as in previous years. Each treatment was used with and without presprinkling. Having concluded that presprinkling produced sufficient increase in effectiveness of control, this procedure was used with all treatments in 1926.

EXPERIMENTAL DATA FOR 1925

A series of 31 treatments was used for the main test, including two untreated checks. Each treatment was replicated three times, each replication consisting of 100 seed pieces, averaging 2 to 3 ounces in weight, planted in a 120-foot row. Each row was harvested separately, and averages for the three replications were computed. Percentages were based on both weights

¹ Approved for publication by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 45.

² Raeder, J. M., C. W. Hungerford, and Naomi Chapman. Seed treatment control of rhizoctonia in Idaho. Idaho Agr. Exp. Sta. Res. Bul. 4. 1925.

and numbers. Average percentages were based on the average production of the three replications for each treatment.

In tabulating results, the tubers from each row were divided into four groups, namely, clean marketable tubers, diseased marketable tubers, clean culls and diseased culls. The division between marketable tubers and culls was based upon size. All tubers over one and three-fourth inches in diameter were considered marketable. Tubers showing even a single sclerotium were considered diseased. The Netted Gem variety of potatoes was used in these trials.

In tabulating results, both weight and number of tubers were listed for each classification. Percentages were based on both tabulations. It is interesting to note that when both methods are used the results are quite similar. This holds true for either the total percentage of control or total percentage infected. Such is not the case for the smaller divisions, as for example, percentage of clean marketable tubers, clean culls, etc.

Table 1 gives the list of treatments used in 1925. It also gives the yields for each replication of each treatment, the percentage of control secured for each replication of each treatment, and the total percentage of control.

Best control was obtained with Semcesan when applied at the rate of 2 ounces per bushel to presprinkled seed, although the control obtained with the same dust when applied at the rate of 3 ounces per bushel on presprinkled seed was nearly as good. The former gave 98.5 per cent control on the basis of weights and 98.3 per cent on the basis of numbers. The latter

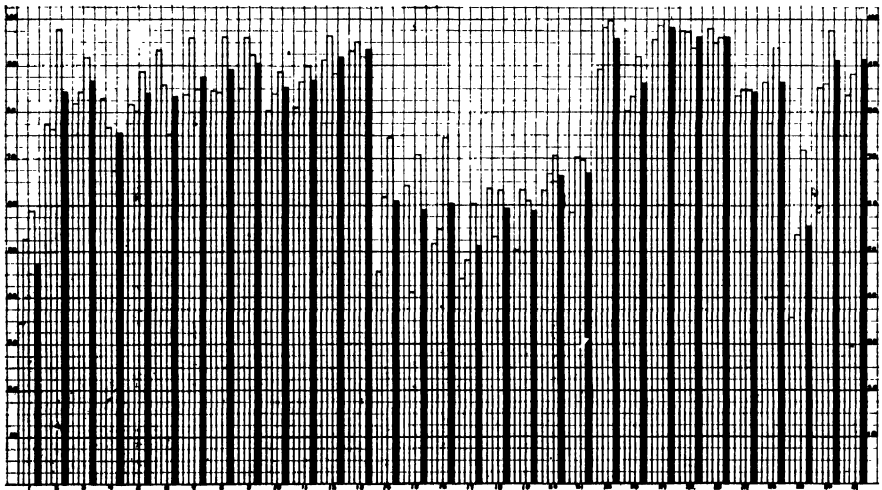


FIG. 1. Percentages of clean tubers by weight secured by various seed treatments (table 1) for the control of rhizoctonia of potatoes in 1925 at Moscow, Idaho.

Three replications and average for each treatment.

produced 96.1 per cent control on the basis of weights and 96.2 per cent on the basis of numbers.

Contrary to expectations, Semesan, in 1925, surpassed Dupont Dust No. 15 in effectiveness. This was not the case in 1924. Hot formalin when used at a temperature of 50° C. for four minutes was quite uniform in its control both years, producing 82.7 per cent control by weight and 80.5 per cent control by number in 1924, and 81.3 per cent control by weight and 84.0 per cent control by number in 1925.

Figure 1 gives in graphic form the results obtained in 1925 for each replication and the average of each treatment when the percentages were based on weights. Figure 2 gives the results when based on numbers, and figure 3 shows a comparison of the average controls obtained by both the above methods.

Stand counts were made in 1925. Table 2 gives in detail the results of these counts. Upon examining this table, it will be noticed that the furfural treatments were quite injurious to germination. This is particularly true with presprinkled seed. This factor in itself would necessarily eliminate this chemical from the list of desirable potato seed treating compounds. All other counts showed over 90 per cent stand except that on plants treated with Semesan when applied to presprinkled seed at the rate of 3 ounces per bushel.

Because the results obtained in 1924 with Dupont Dust No. 15 were so satisfactory, a series of tests with this disinfectant was conducted in 1925 in comparison with the standard mercuric chloride and hot formalin method.

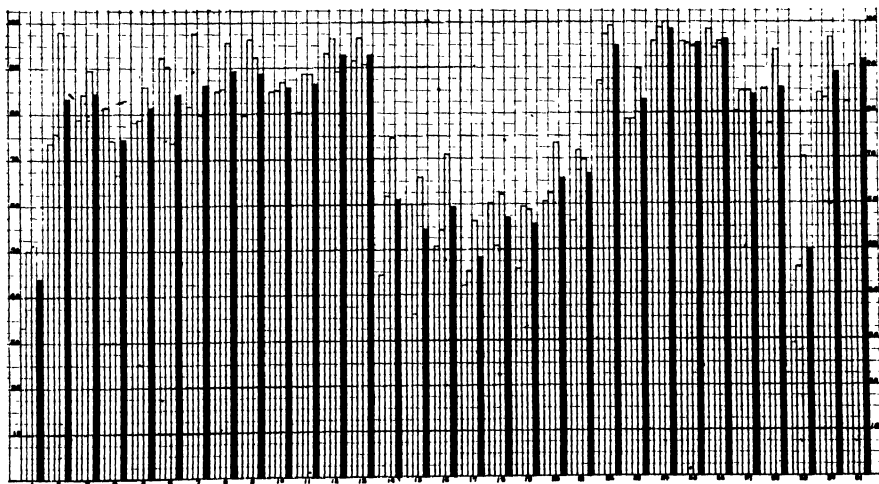


FIG. 2. Percentages of clean tubers by number secured by various seed treatments (table 1) for the control of rhizoctonia of potatoes in 1925 at Moseow, Idaho.
Three replications and average for each treatment.

TABLE 1.—The effectiveness of various seed treatments in controlling rhizoctonia of potatoes in 1935 at Moscow, Idaho

	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
				Clean					Diseased					Clean			Diseased		
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
1. Cold soak (10 treatment)	1	33.3	34.6	11.5	23.2	59	16.2	23.5	47.5	119	32.6	5.0	10.1	67	18.4	9.5	19.1	120	32.9
	2	50.0	52.7	8.0	22.9	33	11.9	10.0	28.6	47	16.9	9.5	27.1	113	40.8	7.5	21.4	84	30.3
	3	51.2	58.9	12.0	29.3	57	21.5	15.0	36.6	61	23.0	9.0	21.9	99	37.4	5.0	12.2	48	18.1
	Ave.	43.8	47.2	10.5	25.1	49.7	16.4	16.2	38.8	75.7	25.0	7.8	18.7	93.0	30.8	7.3	17.5	84	27.8
2. Cold formalin (1-240), 1½ hrs. soak	1	73.6	77.3	11.0	25.3	48	13.2	5.5	12.6	23	6.3	21.0	48.3	234	64.1	6.0	13.8	60	16.4
	2	75.7	76.4	21.5	58.1	96	40.3	7.0	18.9	31	13.0	6.5	17.6	86	36.1	2.0	5.4	25	10.5
	3	98.0	97.8	39.0	78.0	187	56.8	1.0	2.0	7	2.1	10.0	20.0	135	41.0	0.0	0.0	0	0.0
	Ave.	83.4	84.3	23.8	54.7	110.3	35.5	4.5	10.3	20.3	6.5	12.5	28.7	151.7	48.8	2.7	6.2	28.3	9.1
3. do 1 resprinkled	1	78.9	81.9	19.0	42.2	77	29.1	7.5	16.7	30	11.3	16.5	36.7	140	52.8	2.0	4.4	18	6.8
	2	84.3	84.3	21.5	51.8	83	34.3	4.5	10.8	19	7.9	13.5	32.5	121	50.0	2.0	4.8	19	7.9
	3	89.5	91.7	21.0	44.2	93	26.3	3.5	7.4	15	4.2	11.5	45.3	231	65.4	1.5	3.2	14	3.9
	Ave.	84.4	86.6	20.5	45.9	84.3	29.4	5.2	11.6	21.3	7.4	17.2	38.5	164.0	57.2	1.8	4.0	17.0	5.9
4. Formalin (1-120), 50° C., 4 min.	1	81.4	83.0	26.5	54.6	110	35.4	7.0	14.4	28	9.0	13.0	26.8	148	47.6	2.0	4.1	25	8.0
	2	74.1	76.7	17.5	43.2	73	28.9	7.5	18.5	26	10.4	12.5	30.9	121	47.8	3.0	7.4	33	13.0
	3	66.7	67.4	16.5	35.5	71	21.3	9.0	19.4	37	11.1	14.5	31.2	154	46.1	6.5	13.9	72	21.6
	Ave.	74.3	75.5	20.2	44.8	84.7	28.3	7.8	17.3	30.3	10.1	13.3	29.5	141.0	47.2	3.8	8.4	43.3	14.5
5. do presprinkled	1	78.4	81.6	25.0	49.0	110	31.5	8.0	15.7	31	8.9	15.0	29.4	175	50.1	3.0	5.9	33	9.5
	2	78.8	80.2	20.5	57.7	103	38.6	5.5	13.5	26	9.7	7.5	21.1	111	41.6	2.0	5.6	27	10.1
	3	86.0	88.7	26.0	52.0	137	35.3	6.0	12.0	30	7.7	17.0	34.0	207	53.4	1.0	2.0	14	3.6
	Ave.	81.3	84.0	23.8	52.3	116.7	34.9	6.5	14.3	29.0	8.7	13.2	29.0	164.3	49.1	2.0	4.4	24.7	7.4
6. Formalin (1-120), 55° C., 4 min.	1	92.3	93.4	22.5	57.7	79	34.5	2.5	6.4	10	4.4	13.5	34.6	135	58.9	0.5	1.3	5	2.2
	2	90.4	85.8	33.5	71.3	137	47.4	3.0	6.4	13	4.5	9.0	19.1	111	38.4	1.5	3.2	28	9.7
	3	73.6	75.2	32.0	56.1	146	40.7	11.5	20.2	52	14.5	10.0	17.5	124	34.5	3.5	6.1	37	10.3
	Ave.	84.3	83.4	29.3	61.6	120.7	41.3	5.7	11.9	25.0	8.6	10.8	22.7	123.3	42.1	1.8	3.8	23.3	7.9
7. do presprinkled	1	81.6	83.9	19.5	51.3	84	33.9	5.5	14.5	23	9.3	11.5	30.3	124	50.0	1.5	3.9	17	6.9
	2	97.7	95.9	32.0	74.4	138	51.7	0.5	1.2	3	1.1	10.0	23.3	118	44.2	0.5	1.2	8	2.9
	3	79.6	84.9	16.5	33.7	68	18.3	6.5	13.3	23	6.2	22.5	45.9	247	66.6	3.5	7.1	33	8.9
	Ave.	86.2	87.9	22.7	52.3	96.7	32.7	4.2	9.7	16.3	5.5	14.7	33.9	163.0	55.2	1.8	4.1	19.3	6.5
8. Furfural (1-60), 10 min., 50° C.	1	84.9	84.7	24.0	55.8	107	37.9	4.5	10.5	20	7.1	12.5	29.1	132	46.8	2.0	4.7	23	8.2
	2	85.5	84.3	27.5	61.1	129	40.4	3.5	7.8	16	5.0	11.0	24.4	140	43.9	3.0	6.7	34	10.7
	3	95.6	96.2	31.0	55.4	134	36.6	2.0	3.6	9	2.5	22.5	40.2	218	59.6	0.5	0.9	5	1.4
	Ave.	89.3	89.0	27.5	57.4	123.3	38.3	3.3	6.9	15.0	4.7	15.3	31.9	163.3	50.7	1.8	3.8	20.7	6.4

TABLE 1.—(Continued)

Treatment	Plot	Marketable tubers										Culls			
		Clean					Diseased					Clean		Diseased	
		Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Total control in per cent by wt.	Total control in per cent by no.	Wt. ^a	%	No.	%
9. Furfural (1-60), 10 min., 50° C., presprinkled	1	26.0	59.1	124	45.4	8.0	18.2	31	11.4	79.6	85.0	9.0	20.5	108	39.6
	2	22.0	81.5	78	61.9	0.5	1.9	3	2.4	96.3	96.0	4.0	14.8	43	34.1
	3	26.0	50.0	102	30.5	3.0	5.8	9	2.7	92.4	92.4	22.0	42.3	207	61.9
	Ave.	24.7	60.2	101.3	41.5	3.8	9.3	14.3	5.9	88.7	90.4	11.7	28.5	119.3	48.9
10. Furfural (1-120), 10 min., 55° C.	1	23.5	65.3	86	42.6	4.0	11.1	19	9.4	84.7	80.2	7.0	19.4	76	37.6
	2	27.5	68.8	112	47.1	5.0	12.5	23	9.7	85.1	84.0	6.5	16.3	88	36.9
	3	31.5	58.3	147	41.2	6.0	11.1	25	7.0	87.0	88.3	13.5	28.7	170	47.6
	Ave.	27.5	63.4	115.0	43.3	5.0	11.5	22.3	8.4	85.8	85.2	9.7	22.4	111.3	41.9
11. do presprinkled	1	14.5	63.0	66	46.5	3.5	15.2	15	10.6	80.4	81.0	4.0	17.4	49	34.5
	2	17.5	66.0	69	44.2	1.5	5.7	5	3.2	88.6	86.5	6.0	22.6	66	42.3
	3	25.0	63.3	118	44.0	3.5	8.9	16	5.9	88.6	89.9	10.0	25.3	123	45.9
	Ave.	19.0	63.9	84.3	44.7	2.8	9.4	12.0	6.4	86.5	86.7	6.7	22.6	79.3	42.0
12. Mercuric chloride (1-1,000), 1½ hrs. soak	1	28.5	78.1	124	60.2	2.0	5.5	12	5.8	93.2	91.3	5.5	15.1	64	31.1
	2	30.5	71.8	135	50.0	1.0	2.4	4	1.5	96.6	95.0	1.5	10.5	125	46.3
	3	28.0	60.9	110	38.5	3.0	6.5	12	4.2	89.2	88.2	13.0	28.3	142	49.7
	Ave.	29.0	69.5	123.0	48.4	2.0	4.8	9.3	3.7	92.8	91.8	9.7	23.3	110.3	43.4
13. do presprinkled	1	25.0	53.2	96	32.0	3.0	6.4	10	3.3	91.5	93.3	18.0	38.3	184	61.3
	2	27.5	61.8	118	39.9	0.5	1.1	4	1.4	96.6	95.0	1.5	15.5	163	55.1
	3	30.5	70.1	134	51.3	3.0	6.9	14	5.4	90.8	91.9	9.0	20.7	106	40.6
	Ave.	27.7	61.4	116.0	40.6	2.2	4.9	9.3	3.3	92.9	93.5	14.2	31.5	151.0	52.9
14. Check (no treatment)	1	14.0	31.1	63	19.4	18.0	40.0	86	26.5	44.4	45.6	6.0	13.3	85	26.2
	2	62.4	61.8	80	21.6	10.0	21.5	51	13.7	62.4	61.8	11.0	23.7	149	40.2
	3	74.8	29.3	53	15.2	6.0	12.1	25	7.2	74.8	74.5	22.5	45.5	207	59.3
	Ave.	15.5	32.9	65.3	18.7	11.3	24.0	54.0	15.5	61.0	60.9	13.2	28.1	147.0	42.2
15. Dupont dust No. 15, 1 oz. per bu.	1	16.0	40.0	75	26.6	12.5	31.3	56	19.9	60.0	64.2	8.0	20.0	106	37.6
	2	35.9	18.5	40	12.2	20.5	44.6	88	26.8	35.9	41.1	8.0	17.4	95	28.9
	3	66.0	44.9	114	30.6	15.0	27.5	63	16.9	66.0	70.8	11.5	21.1	150	40.2
	Ave.	54.5	34.8	76.3	23.3	16.0	34.2	69.0	21.1	54.5	59.0	9.2	19.7	117.0	35.7
16. do presprinkled	1	12.0	32.9	52	20.8	11.5	31.5	51	20.4	50.7	51.6	6.5	17.8	77	30.8
	2	54.5	38.0	101	25.9	16.0	31.7	82	21.0	54.5	54.8	8.0	15.8	113	28.9
	3	71.0	30.6	54	17.6	8.0	16.0	30	9.8	71.0	74.5	20.5	41.0	174	56.9
	Ave.	15.5	33.9	69.0	21.8	11.8	25.8	54.3	17.2	59.5	60.3	11.7	25.6	121.3	38.5

TABLE 1.—(Continued)

Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
			Clean					Diseased					Clean			Diseased		
			Wt.*	%	No.	%	Wt.*	%	No.	%	Wt.*	%	Wt.*	%	No.	%	No.	%
17. Dupont dust No. 15, 3 oz. per bu.	1	42.0	13.0	29.5	61	18.4	19.5	44.3	98	29.6	5.5	12.5	85	25.7	6.0	13.6	87	26.3
	2	48.1	11.5	28.0	54	17.8	15.0	36.6	76	25.1	7.0	17.1	92	30.4	7.5	18.3	81	26.7
	3	56.4	18.0	35.6	72	21.4	14.0	27.7	50	14.9	10.5	20.8	131	38.9	8.0	15.8	83	24.7
	Ave.	48.2	14.2	31.3	62.3	19.3	16.2	35.8	74.7	23.1	7.7	16.9	102.7	31.8	7.2	15.9	83.7	25.9
18. do presprinkled	1	60.2	12.5	34.2	60	20.7	8.5	23.3	39	13.4	9.5	26.0	124	42.8	6.0	16.4	67	23.1
	2	50.8	15.5	26.7	64	15.6	19.5	33.6	87	21.2	14.0	24.1	154	37.5	9.0	15.5	106	25.8
	3	62.1	12.5	26.3	52	13.8	8.0	16.8	36	9.6	17.0	35.8	186	49.5	10.0	21.1	102	27.1
	Ave.	57.0	13.5	28.5	58.7	16.3	12.0	25.4	54.0	15.0	13.5	28.5	154.7	43.1	8.3	17.5	91.7	25.5
19. Dupont dust No. 15, 3 oz. per bu.	1	45.8	11.0	26.5	50	17.1	16.0	38.6	68	23.2	8.0	19.3	98	33.4	6.5	15.7	77	26.3
	2	59.5	21.5	40.6	106	29.7	15.0	28.3	67	18.8	10.0	18.9	120	33.6	6.5	12.3	64	17.9
	3	58.6	61.0	48.3	141	38.9	19.5	33.6	86	23.8	6.0	10.3	80	22.1	4.5	7.8	55	15.2
	Ave.	55.5	20.2	39.8	99.0	29.4	16.8	33.1	73.7	21.8	8.0	15.7	99.3	29.4	5.8	11.4	65.3	19.4
20. do presprinkled	1	60.5	24.0	44.0	122	31.1	16.5	30.3	82	20.9	9.0	16.5	126	32.1	5.0	9.2	62	15.8
	2	62.5	17.5	39.8	87	27.0	12.5	28.4	66	20.5	10.0	22.7	128	39.8	4.0	9.1	41	12.7
	3	73.4	20.5	43.6	85	26.3	6.5	13.8	30	9.3	14.0	29.8	143	44.3	6.0	12.8	65	20.1
	Ave.	65.7	20.7	42.9	98.0	28.2	11.5	23.9	61.3	17.6	11.0	22.8	132.3	38.1	5.0	10.4	56.0	16.1
21. Semesan dust, 1 oz. per bu.	1	56.4	16.5	42.3	84	30.3	13.0	33.3	65	23.5	5.5	14.1	78	28.2	4.0	10.3	50	18.1
	2	71.8	20.0	38.8	87	24.7	6.5	12.6	27	7.7	17.0	33.0	161	45.7	8.0	15.5	77	21.9
	3	69.6	27.0	52.9	113	34.9	12.0	23.5	54	16.7	8.5	16.7	112	34.7	3.5	6.9	44	13.6
	Ave.	66.7	21.2	44.9	94.7	29.8	10.5	22.2	48.7	15.3	10.3	21.8	117.0	36.9	5.2	11.0	57.0	17.9
22. do presprinkled	1	87.0	26.5	56.9	115	38.9	5.0	10.8	21	7.1	14.0	30.1	149	50.3	1.0	2.2	11	3.7
	2	97.3	37.5	66.9	168	48.1	1.0	1.8	3	0.9	17.0	30.4	175	50.1	0.5	0.9	3	0.9
	3	99.1	36.5	71.6	150	48.2	0.5	0.9	1	0.3	14.0	27.5	160	51.4	0.0	0.0	0	0.0
	Ave.	94.7	33.5	65.4	144.3	45.3	2.2	4.3	8.3	2.6	15.0	29.3	161.3	50.6	0.5	1.0	4.7	1.5
23. Semesan dust, 2 oz. per bu.	1	78.6	20.0	53.3	99	35.5	6.0	16.0	26	9.4	9.5	25.3	124	44.6	2.0	5.3	29	10.4
	2	78.7	27.0	57.4	143	44.5	9.0	19.1	40	12.5	10.0	21.3	125	38.9	1.0	2.1	13	4.0
	3	89.6	23.0	43.4	105	24.7	3.5	6.6	15	3.5	24.5	46.2	286	67.3	2.0	3.8	19	4.5
	Ave.	82.8	23.3	50.8	115.7	33.9	6.2	13.5	27.0	7.9	14.7	32.0	178.3	52.2	1.7	3.7	20.3	5.9
24. do presprinkled	1	95.9	27.5	74.3	110	52.6	1.0	2.7	6	2.9	8.0	21.6	90	43.1	0.5	1.4	3	1.4
	2	98.9	39.0	84.8	191	66.8	0.0	0.0	0	0.0	6.5	14.1	91	31.8	0.5	1.1	4	1.4
	3	100.0	34.0	79.1	142	55.0	0.0	0.0	0	0.0	9.0	20.9	116	44.9	0.0	0.0	0	0.0
	Ave.	98.5	33.5	79.9	147.6	58.8	0.3	0.7	2.0	0.8	7.8	18.6	99.0	39.5	0.3	0.7	2.3	0.9

TABLE I—(Concluded)

Treatment	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls							
				Clean					Diseased					Clean				Diseased			
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%		
25. Semesan dust, 3 oz. per bu.	1	95.7	97.5	18.0	51.4	74	30.6	1.0	1.2	3	2.9	15.5	44.3	162	66.9	0.5	1.4	3	1.2		
	2	95.3	97.2	24.0	55.8	140	43.8	1.5	1.6	5	3.5	17.0	39.5	171	53.4	0.5	1.2	4	1.3		
	3	94.7	93.8	26.5	69.7	121	47.5	1.5	2.7	7	3.9	9.5	25.0	118	46.3	0.5	1.3	9	3.5		
	Ave.	95.4	96.2	22.8	59.1	111.7	41.0	1.3	1.8	5.0	3.4	14.0	36.3	150.3	55.2	0.5	1.3	5.3	1.9		
26. do presprinkled	1	98.5	98.0	22.5	65.2	86	42.8	0.5	0.9	2	1.4	11.5	33.3	111	55.2	Tr.	Tr.	2	0.9		
	2	94.3	94.8	35.0	80.5	144	61.8	2.0	4.6	9	3.9	6.0	13.8	77	33.0	0.5	1.1	3	1.3		
	3	95.7	96.0	35.0	75.3	147	53.5	1.5	3.2	5	3.2	9.5	20.4	117	42.5	0.5	1.1	6	2.2		
	Ave.	96.1	96.2	30.8	74.4	125.7	53.2	1.3	3.1	5.3	3.1	2.2	21.7	101.7	43.0	0.3	0.7	3.7	1.6		
27. Bayer dust, 2 oz. per bu., presprinkled	1	80.3	83.5	18.5	48.7	77	27.0	0.4	10.5	17	5.9	12.0	31.6	161	56.5	3.5	9.2	30	10.5		
	2	84.8	84.8	21.0	42.4	77	26.0	4.5	9.1	18	6.1	21.0	42.4	174	58.8	3.0	6.1	27	9.1		
	3	84.7	84.7	27.5	56.1	127	34.8	5.5	11.2	26	7.1	14.0	28.6	182	49.9	2.0	4.1	30	8.2		
	Ave.	83.9	84.3	22.3	49.2	93.7	29.7	4.5	9.9	20.3	6.4	15.7	34.7	172.3	54.6	2.8	6.2	29.0	9.2		
28. Bayer dust, 3 oz. per bu., presprinkled	1	85.3	86.3	21.0	51.2	104	32.4	4.5	10.9	22	6.9	14.0	34.1	173	53.9	1.5	3.7	22	6.9		
	2	77.7	77.5	27.5	53.4	127	37.0	7.5	14.6	33	9.6	12.5	24.3	139	40.5	4.0	7.8	44	12.8		
	3	93.8	94.3	25.5	52.6	127	33.1	1.5	3.1	6	1.6	20.0	41.2	235	61.2	1.5	3.1	16	4.2		
	Ave.	85.5	86.4	24.7	52.6	119.3	34.2	4.5	9.6	20.3	5.8	15.5	32.9	182.3	52.2	2.3	4.9	27.3	7.8		
29. Check (no treatment)	1	29.2	35.7	5.0	12.7	23	7.2	15.5	39.2	69	21.6	6.5	16.5	91	28.5	12.5	31.6	136	42.6		
	2	46.0	53.6	9.0	15.9	40	9.1	17.5	30.9	80	18.2	17.0	30.1	196	44.5	13.0	23.0	124	28.2		
	3	70.3	71.8	19.0	37.6	96	21.9	9.0	17.8	47	10.8	16.5	32.7	218	49.9	6.0	11.9	76	17.4		
	Ave.	49.8	55.5	11.0	22.5	53.0	13.3	14.0	28.7	65.3	16.4	13.3	27.3	168.3	42.2	10.5	21.5	112.0	28.1		
30. Clean seed, untreated	1	84.3	85.3	23.5	56.6	112	38.4	5.0	12.0	26	8.9	11.5	27.7	137	46.9	1.5	3.6	17	5.8		
	2	83.1	86.0	21.0	44.2	95	27.1	5.0	10.5	18	5.1	18.5	38.9	206	58.9	3.0	6.3	31	8.9		
	3	96.5	97.5	23.0	38.9	108	19.8	1.0	1.7	4	0.7	34.0	57.6	494	77.7	1.0	1.7	10	1.8		
	Ave.	88.8	91.1	22.5	45.6	105.0	26.5	3.7	7.5	16.0	4.0	21.3	43.2	255.7	64.6	1.8	3.7	19.3	4.9		
31. Clean seed, mercuric chloride (1-1,000), 1½ hrs. soak, presprinkled	1	82.2	83.6	20.0	54.8	94	36.9	4.5	12.3	20	7.8	10.0	27.4	119	46.7	2.0	5.5	22	8.6		
	2	90.5	88.1	36.5	76.8	166	59.7	3.0	6.3	16	5.8	6.5	13.7	79	28.4	1.5	3.2	17	6.1		
	3	100.0	100.0	20.0	42.1	91	26.9	0.0	0.0	0	0.0	27.5	57.9	247	73.1	0.0	0.0	0	0.0		
	Ave.	91.6	91.4	25.5	58.1	117.0	40.3	2.5	5.7	12.0	4.1	14.7	33.5	148.3	51.1	1.2	2.7	13.0	4.5		

^a Weight in lbs.

Table 3 gives in detail the treatments used and results obtained with various amounts of this compound. The highest percentages of control with the dust treatments were obtained when the dust was applied at the rate of 4 ounces per bushel to presprinkled cut seed: 81.1 per cent control by weight, and 80.6 per cent control by number. This did not equal the control obtained with either mercuric chloride or hot formalin, nor the control obtained with Dupont Dust in 1924. All of the tubers in this particular series of treatments, except the checks, were presprinkled before receiving further treat-

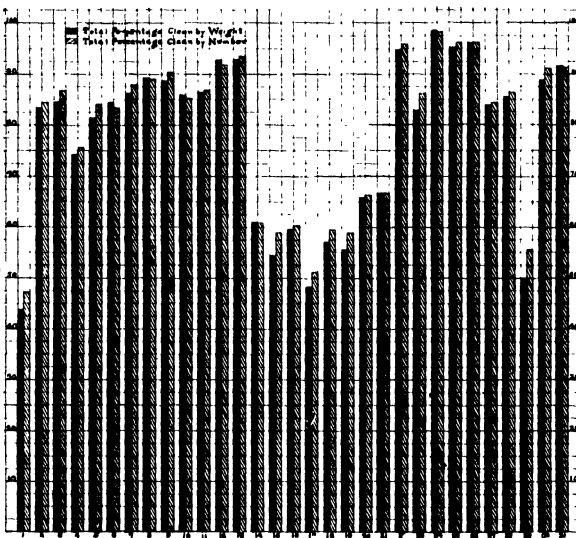


FIG. 3 (Left). Comparison of the average percentages of clean tubers by weight and by number secured by various seed treatments (table 1) for the control of rhizoctonia of potatoes in 1925 at Moscow, Idaho.

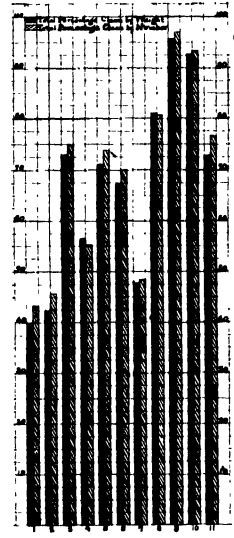


FIG. 4 (Right). Comparison of the average percentages of clean tubers by weight and by number secured by treatment with Dupont Dust No. 15 (table 3) for the control of rhizoctonia of potatoes in 1926 at Moscow, Idaho.

ments. The Early Ohio variety of potatoes was used in these tests. Figure 4 gives in a graphic manner the results of this experiment.

EXPERIMENTAL DATA FOR 1926

A somewhat more extensive group of tests was made in 1926, the general procedure of which closely approximated that of former years. More checks, however, were included in the list, as was also a more extensive list of disinfectants.

Each treatment was replicated three times, each replication consisting of 50 seed pieces, of from 2 to 3 ounces in weight, planted in a 60-foot row.

TABLE 3.—*The effectiveness of Dupont dust No. 15 as compared with mercuric chloride and formalin treatments for the control of rhizoctonia of potatoes in 1925 at Moscow, Idaho*

Treatment	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers						Culls					
			Clean			Diseased			Clean			Diseased		
			Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
1. Check (no treatment) —	39.6	43.4	35.0	28.3	132	21.7	57.5	46.6	198	32.5	14.0	11.3	132	21.7
2. Dupont dust No. 15, 1 oz. per bu. Cut before treating	42.3	45.8	40.5	29.3	115	18.8	62.5	45.3	182	29.7	18.0	13.0	165	27.0
3. Dupont dust No. 15, 1 oz. per bu. Cut after treating	72.9	74.8	91.0	56.0	278	41.2	37.0	22.8	110	16.3	27.5	16.9	227	33.6
4. Dupont dust No. 15, 2 oz. per bu. Cut before treating	56.4	55.1	78.0	46.3	215	31.6	59.5	35.3	175	25.7	17.0	10.1	160	23.5
5. Dupont dust No. 15, 2 oz. per bu. Cut after treating	71.0	73.8	94.0	54.7	262	35.3	40.0	23.3	110	14.8	28.0	16.3	286	38.5
6. Dupont dust No. 15, 3 oz. per bu. Cut before treating	67.3	70.0	89.0	57.6	289	46.0	43.5	28.2	127	20.2	15.0	9.7	151	24.0
7. Dupont dust No. 15, 3 oz. per bu. Cut after treating	47.8	48.4	49.0	35.8	157	25.2	54.5	39.8	178	28.5	16.5	12.0	145	23.2
8. Dupont dust No. 15, 4 oz. per bu. Cut before treating	81.1	80.6	97.5	67.0	306	50.0	20.5	14.1	58	9.5	20.5	14.1	187	30.6
9. Mercuric chloride (1-1,000), 1½ hrs. soak	95.8	97.0	113.0	73.4	319	49.8	5.5	3.6	14	2.2	34.5	22.4	302	47.2
10. Formalin (1-120), 55° C., 3 min.	92.6	93.3	101.0	74.2	309	52.8	8.0	5.9	25	4.3	25.0	18.4	237	40.5
11. Check (no treatment) —	72.8	76.5	58.5	47.6	198	30.8	26.5	21.5	81	12.4	31.0	25.2	294	45.7

^a Weight in lbs.

Tabulation of results was the same as in former years. The Netted Gem variety of potatoes was again used. All tubers were presprinkled.

Table 4 gives the list of treatments, the product of each replication, and percentages of control. In examining table 4 it will be noticed that the best control in 1926 was obtained with formalin, when presprinkled potatoes were dipped in a 1-120 solution, at 125° F., for four minutes.

Figure 5 depicts in a graphic manner the average percentages of control,

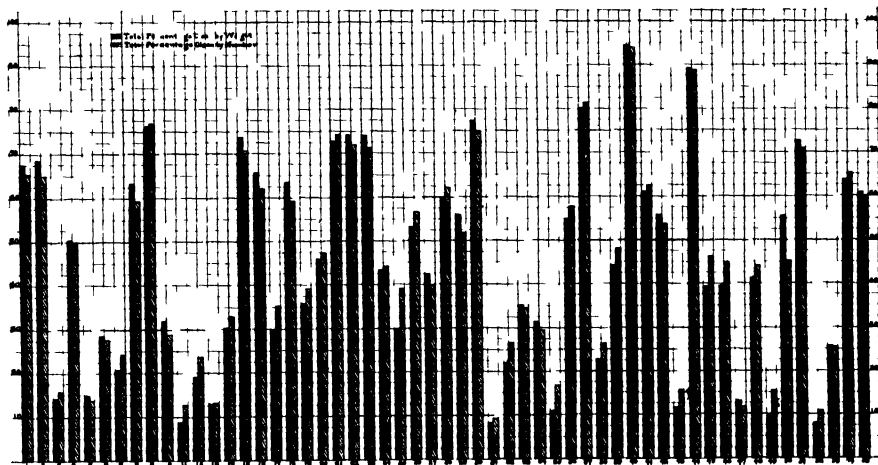


FIG. 5. Comparison of the average percentages of clean tubers by weight and by number secured by various seed treatments (table 4) for the control of rhizoctonia of potatoes in 1926 at Moscow, Idaho.

of the 1926 tests, based on number and on weight. A comparison of the data seems to indicate that percentages of control are comparable when based on either weights or numbers.

DISCUSSION

It would seem desirable to compare more closely results obtained by the same treatment for the various years. With that in mind, table 5 was prepared. The table includes results obtained in 1924, which are already in published form.

In examining the table it will be noticed that the checks reacted quite similarly each year: all three types of checks were most free from infection in 1925, less free in 1924, and least free from infection in 1926. This is not exactly the case with the checks of clean seed treated with mercuric chloride, as the percentage of control with these checks was the same in 1924 and 1926.

The results with tubers treated with mercuric chloride followed the same curve as did the results of the checks for the three years: they were freer from infection in 1925, when the checks were most free; least free in 1926

TABLE 4.—(Continued)

Treatment	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
				Clean					Diseased					Clean			Diseased		
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
9. Check (clean seed), mercuric chloride, (1-1,000), 1½ hrs.	1	35.4	33.9	18.0	32.7	42	22.6	29.0	52.7	63	33.9	1.5	2.7	21	11.3	6.5	11.8	60	32.2
	2	94.9	92.9	71.5	89.9	192	72.2	3.5	4.4	11	4.1	4.0	5.0	55	20.7	0.5	0.6	8	3.0
	3	88.3	93.3	48.0	80.0	113	63.1	7.0	11.7	12	6.7	5.0	8.3	54	30.2	0.0	0.0	0	0.0
	Ave.	76.1	76.8	45.8	70.7	115.7	55.9	13.2	20.4	25.3	12.2	3.5	5.4	43.3	20.9	2.3	3.4	22.7	10.9
10. Check (diseased seed)	1	22.3	16.5	11.0	20.4	20	11.0	34.0	62.9	81	44.7	1.0	1.9	10	5.5	8.0	14.8	70	38.7
	2	38.4	38.7	19.0	30.4	42	18.7	30.5	48.8	69	30.7	5.0	8.0	45	20.0	8.0	12.8	69	30.7
	3	34.3	28.7	17.0	32.4	45	22.3	27.5	52.4	72	35.6	1.0	1.9	13	6.4	7.0	13.3	72	35.6
	Ave.	31.9	28.8	15.7	27.8	35.7	17.6	30.7	54.4	74.0	36.5	2.3	4.1	22.7	11.2	7.7	13.7	70.3	34.7
11. Dupont dust No. 12, 2 oz. per bu., before cutting	1	10.3	22.6	4.0	7.5	14	7.7	43.0	80.4	101	55.8	1.5	2.8	27	14.9	5.0	9.3	39	21.5
	2	13.9	15.6	9.0	13.2	23	9.7	54.0	79.4	141	59.2	0.5	0.7	14	5.9	4.5	6.6	60	25.2
	3	0.9	1.8	0.5	0.9	4	1.8	52.5	93.8	165	72.7	0.0	0.0	0	0.0	3.0	5.4	58	25.6
	Ave.	8.8	12.6	4.5	7.6	13.7	6.4	49.8	84.1	135.7	63.1	0.7	1.2	13.3	6.2	4.2	7.1	52.3	24.3
12. Dupont dust No. 12, 3 oz. per bu., after cutting	1	9.1	17.5	5.0	8.3	21	9.7	53.0	87.6	145	66.8	0.5	0.8	17	7.8	2.0	3.3	34	15.7
	2	33.6	38.8	18.0	28.8	46	20.3	36.5	58.4	79	34.8	3.0	4.8	42	18.5	5.0	8.0	60	26.4
	3	13.2	14.4	7.0	12.7	28	12.2	45.0	81.4	139	60.7	0.3	0.5	5	2.2	3.0	5.4	57	24.9
	Ave.	19.0	23.6	10.0	16.8	31.7	14.1	44.8	75.4	121.0	53.9	1.3	2.2	21.3	9.5	3.3	5.6	50.3	22.4
13. Dupont dust No. 12, 10% solution, dip	1	4.7	2.7	3.0	4.7	5	2.7	54.0	85.0	121	65.1	0.0	0.0	0	0.0	6.5	10.2	60	32.3
	2	4.6	2.4	3.0	4.6	5	2.4	54.0	83.1	130	62.8	0.0	0.0	0	0.0	8.0	12.3	72	34.8
	3	29.1	31.7	17.0	28.3	52	23.9	41.5	69.2	117	53.7	0.5	0.8	17	7.8	1.0	1.7	32	14.7
	Ave.	12.9	13.0	7.7	12.6	20.7	10.2	47.8	78.5	122.7	60.2	0.2	0.3	5.7	2.8	5.2	8.5	54.7	26.8
14. Dupont dust No. 37, 2 oz. per bu., before cutting	1	32.8	31.4	18.5	29.6	50	24.9	38.5	61.6	96	47.8	2.0	3.2	13	6.5	3.5	5.6	42	20.8
	2	29.0	32.8	15.5	23.1	34	17.4	44.0	65.7	99	50.8	4.0	5.9	30	15.4	3.5	5.2	32	16.4
	3	28.7	34.3	12.5	26.6	44	21.6	29.5	62.8	76	37.3	1.0	2.1	26	12.7	4.0	8.5	58	28.4
	Ave.	30.3	32.9	15.5	26.4	42.7	21.4	37.3	63.4	90.3	45.2	2.3	3.9	23.0	11.5	3.7	6.3	44.0	22.0
15. Check (clean seed)	1	78.0	75.8	52.5	70.0	123	55.2	14.0	18.7	32	14.3	6.0	8.0	46	20.6	2.5	3.3	22	9.9
	2	74.5	69.6	40.0	60.2	99	38.9	11.5	17.3	32	12.6	9.5	14.3	78	30.7	5.5	8.2	45	17.7
	3	67.2	65.5	34.0	58.6	76	39.2	18.5	31.9	48	24.7	5.0	8.6	51	26.3	0.5	0.9	19	9.8
	Ave.	73.7	70.5	42.2	63.5	99.3	44.4	14.7	22.1	37.3	16.7	6.8	10.2	55.3	26.1	2.8	4.2	28.7	12.8
16. Check (clean seed), mercuric chloride (1-1,000), 1½ hrs.	1	94.2	90.4	52.0	85.9	107	57.2	3.0	4.9	6	3.2	5.0	8.3	62	33.2	0.5	0.8	12	6.4
	2	100.0	100.0	65.5	91.6	153	75.7	0.0	0.0	0	0.0	6.0	8.4	49	24.3	0.0	0.0	0	0.0
	3	16.4	13.7	11.5	13.5	21	7.8	58.0	67.8	115	42.8	2.5	2.9	16	5.9	13.5	15.8	117	43.5
	Ave.	65.5	62.0	43.0	59.3	93.7	42.7	20.3	28.0	40.3	18.4	4.5	6.2	42.3	19.3	4.7	6.5	43.0	19.4

TABLE 4.—(Continued)

	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls							
				Clean					Diseased					Clean				Diseased			
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%		
1	57.7	57.8	27.5	47.4	61	26.3	19.0	32.8	47	20.3	6.0	10.3	73	31.5	5.5	9.5	51	21.9			
2	31.8	33.3	16.0	29.1	46	22.9	34.0	61.8	95	47.3	1.5	2.7	21	10.4	3.5	6.4	39	19.4			
3	1.6	2.7	1.0	1.6	4	2.7	55.5	90.7	62	41.9	0.0	0.0	0	0.0	5.0	7.7	82	55.4			
Ave.	29.6	35.3	14.8	24.9	37.0	19.1	37.2	62.5	68.0	35.1	2.8	4.7	31.3	16.2	4.7	7.9	57.3	29.6			
18. Dupont dust No. 37, 3 oz. per bu., after cutting	1	75.7	74.3	42.0	64.9	91	43.9	14.5	22.4	35	16.9	7.0	10.8	63	30.4	1.3	1.9	18	8.7		
	2	85.8	85.4	56.0	82.9	130	63.1	9.0	13.3	24	11.7	2.0	2.9	46	22.3	0.5	0.7	6	2.9		
	3	32.5	25.6	21.5	29.1	41	17.0	41.0	55.4	96	39.8	2.5	3.4	16	6.6	9.0	12.2	88	36.5		
	Ave.	63.4	59.1	39.8	57.9	87.3	40.0	21.5	31.3	51.7	23.7	3.8	5.5	41.7	19.1	3.6	5.2	37.3	17.1		
19. Dupont dust No. 37, 10% solution, dip	1	45.2	55.2	22.0	38.3	59	27.3	27.5	47.8	62	28.7	4.0	6.9	56	25.9	4.0	6.9	39	18.1		
	2	57.2	61.2	32.5	48.9	83	38.8	27.0	40.6	72	33.6	5.5	8.3	48	22.4	1.5	2.3	11	5.1		
	3	5.4	5.6	3.5	5.4	11	4.7	53.0	82.2	133	57.3	Tr.	Tr.	2	0.9	8.0	12.4	86	37.0		
	Ave.	35.8	39.1	19.3	30.7	51.0	23.1	35.8	57.0	89.0	40.3	3.2	5.1	35.3	16.0	4.5	7.2	45.3	20.5		
20. Semesan, 2 oz. per bu., before cutting	1	56.4	56.6	38.5	46.7	83	39.5	28.0	33.9	55	26.2	8.0	9.7	36	17.1	8.0	9.7	36	17.1		
	2	59.4	61.7	31.0	58.5	83	46.1	21.0	39.6	51	28.3	0.5	0.9	28	15.6	0.5	0.9	18	10.0		
	3	26.3	30.0	19.0	23.8	45	17.3	53.0	66.3	120	46.2	2.0	2.5	33	12.7	6.0	7.5	62	23.8		
	Ave.	45.9	47.4	29.5	41.1	70.3	32.5	34.0	47.4	75.3	34.8	3.5	4.8	32.3	14.9	4.8	6.7	38.7	17.9		
21. Semesan, 3 oz. per bu., after cutting	1	100.0	100.0	51.0	91.1	120	56.9	0.0	0.0	0	0.0	5.0	8.9	91	43.1	0.0	0.0	0	0.0		
	2	100.0	100.0	42.0	93.3	82	74.5	0.0	0.0	0	0.0	3.0	6.7	28	25.5	0.0	0.0	0	0.0		
	3	20.4	30.1	10.0	18.5	27	14.5	38.0	70.3	77	41.4	1.0	1.9	29	15.6	5.0	9.3	53	28.5		
	Ave.	72.7	74.3	34.3	66.9	76.3	45.1	12.3	23.9	25.7	15.2	3.0	5.8	49.3	29.2	1.7	3.3	17.7	10.4		
22. Check (clean seed)	1	93.0	90.4	62.0	77.9	137	54.8	5.0	6.3	16	6.4	12.0	15.1	89	35.6	0.5	0.6	8	3.2		
	2	86.8	85.7	36.0	67.9	90	41.5	6.0	11.3	15	6.9	10.0	18.9	96	44.2	1.0	1.9	16	7.4		
	3	45.7	39.1	32.0	41.8	61	26.8	35.0	45.8	74	32.5	3.0	3.9	28	12.3	6.5	8.5	65	28.5		
	Ave.	74.1	72.0	43.3	62.2	96.0	41.4	15.3	21.9	35.0	15.1	8.3	11.9	71.0	30.6	2.7	3.9	29.7	12.8		
23. Check (clean seed), mercuric chloride (1-1,000), 1½ hrs.	1	100.0	100.0	54.0	78.8	119	51.9	0.0	0.0	0	0.0	14.5	21.2	110	48.0	0.0	0.0	0	0.0		
	2	73.8	73.5	43.0	70.5	123	63.7	13.0	21.3	21	10.9	2.0	3.3	19	9.8	3.0	4.9	30	15.5		
	3	46.3	39.5	29.0	45.9	83	37.7	31.0	49.0	87	39.5	0.3	0.4	4	1.8	3.0	4.7	46	20.9		
	Ave.	74.0	71.3	42.0	65.3	108.3	50.6	14.7	22.9	36.0	16.8	5.6	8.7	44.3	20.7	2.0	3.1	25.3	11.8		
24. Check (diseased seed)	1	18.0	22.7	10.5	17.2	26	13.4	47.0	77.0	109	56.2	0.5	0.8	18	9.3	3.0	4.9	41	21.1		
	2	54.2	57.5	25.0	45.9	57	34.5	21.0	38.5	44	26.7	4.5	8.3	38	23.0	4.0	7.3	26	15.8		
	3	63.8	56.9	27.0	57.4	57	37.7	13.0	27.7	34	22.5	3.0	6.4	29	19.2	4.0	8.5	31	20.5		
	Ave.	43.3	44.1	20.8	38.4	46.7	27.5	27.0	49.8	62.3	36.6	2.7	4.9	28.3	16.6	3.7	6.8	32.7	19.2		

TABLE 4.—(Continued)

Treatment	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
				Clean			Diseased							Clean			Diseased		
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
25. Bayer dust, 2 oz. per bu., before cutting	1	38.3	50.7	21.0	29.8	44	18.3	40.0	56.7	91	37.8	6.0	8.5	78	32.4	3.5	4.9	28	11.6
	2	52.1	54.0	22.5	46.9	59	31.2	20.0	41.7	52	27.5	2.5	5.2	43	22.8	3.0	6.3	35	18.5
	3	4.9	9.8	3.0	4.5	14	7.2	60.0	90.6	131	67.5	0.3	0.4	5	2.6	3.0	4.5	44	22.7
	Ave.	29.9	39.0	15.5	25.2	39.0	18.8	40.0	64.9	91.3	43.9	2.9	4.7	42.0	20.2	3.2	5.2	35.7	17.2
26. Bayer dust, 3 oz. per bu., after cutting	1	58.4	62.1	38.5	47.8	84	30.7	30.0	37.3	68	24.8	8.5	10.6	86	31.4	3.5	4.3	36	13.1
	2	70.3	70.2	38.0	69.4	99	61.5	16.0	29.2	43	26.7	0.5	0.9	14	8.7	0.3	0.5	5	3.1
	3	34.5	38.6	24.0	33.1	64	30.9	44.5	61.4	97	46.9	1.0	1.4	16	7.7	3.0	4.1	30	14.5
	Ave.	53.1	56.6	33.5	48.3	82.3	38.5	30.2	43.6	69.3	32.4	3.3	4.8	38.7	18.1	2.3	3.3	23.7	11.1
27. Colloidal copper, 2 oz. per bu., before cutting	1	71.2	61.7	29.5	67.8	63	38.9	11.0	25.3	34	20.9	1.5	3.4	37	22.8	1.5	3.4	28	17.3
	2	22.7	25.0	3.5	15.9	8	10.5	14.0	63.6	34	44.7	1.5	6.8	11	14.5	3.0	13.6	23	30.3
	3	30.6	30.3	21.0	29.2	54	20.7	47.0	65.3	131	50.2	1.0	1.4	25	9.6	3.0	4.2	51	19.5
	Ave.	42.1	39.7	18.0	39.3	41.7	25.1	24.0	52.4	66.3	39.9	1.3	2.8	24.3	14.6	2.5	5.5	34.0	20.4
28. Colloidal copper, 3 oz. per bu., after cutting	1	70.3	71.3	28.0	69.1	60	52.2	12.0	29.6	33	28.7	0.5	1.2	22	19.1	0.0	0.0	0	0.0
	2	23.0	36.0	2.5	19.2	9	14.1	9.5	73.1	24	37.5	0.5	3.8	14	21.9	0.5	3.8	17	26.6
	3	61.5	69.8	15.5	59.6	35	46.1	10.0	38.5	19	25.0	0.5	1.9	18	23.7	Tr.	Tr.	4	5.3
	Ave.	59.6	62.0	15.3	57.7	34.7	40.8	10.5	39.6	25.3	29.8	0.5	1.9	18.0	21.2	0.2	0.8	7.0	8.2
29. Check (clean seed)	1	81.3	78.1	55.0	76.4	126	52.1	13.0	18.1	41	16.9	3.5	4.9	63	26.0	0.5	0.7	12	4.9
	2	8.3	9.7	6.0	8.3	23	9.7	62.0	85.5	146	61.9	0.0	0.0	0	0.0	4.5	6.2	67	28.4
	3	67.5	63.7	59.0	64.8	150	49.8	26.5	29.1	65	21.6	2.5	2.7	42	13.9	3.0	3.3	44	14.6
	Ave.	55.9	51.9	40.0	53.2	99.7	38.4	30.5	40.6	84.0	32.3	2.0	2.7	35.0	13.5	2.7	3.6	41.0	15.8
30. Check (clean seed), mercuric chloride (1-1,000), 1½ hrs.	1	99.0	99.6	66.0	85.2	127	53.4	0.0	0.0	0	0.0	11.5	14.8	110	46.2	Tr.	Tr.	1	0.4
	2	30.9	26.6	18.5	30.5	44	24.4	41.0	67.5	114	63.3	0.3	0.4	4	2.2	1.0	1.6	18	10.0
	3	92.8	87.0	61.0	87.1	143	63.8	3.0	4.3	10	4.5	4.0	5.7	52	23.2	2.0	2.9	19	8.5
	Ave.	77.1	74.7	48.5	69.5	104.7	48.9	15.0	21.5	41.3	19.3	5.3	7.6	55.3	25.8	1.0	1.4	12.7	5.9
31. Check (diseased seed)	1	13.2	10.6	5.5	10.4	9	5.3	38.0	71.7	82	48.2	1.5	2.8	9	5.3	8.0	15.1	70	41.2
	2	8.3	10.8	5.0	7.9	19	7.9	55.0	87.6	166	68.6	0.3	0.4	7	2.9	2.5	3.9	50	20.7
	3	4.6	6.0	3.0	4.2	7	3.0	61.0	85.6	146	62.9	0.3	0.4	7	3.0	7.0	9.8	72	31.0
	Ave.	8.3	9.1	4.5	7.2	11.7	5.5	51.3	82.3	131.3	61.2	0.7	1.1	7.7	3.6	5.8	9.3	64.0	29.8
32. Colloidal copper, 1% solution, dip	1	42.6	44.4	23.0	40.0	62	25.5	31.0	53.9	88	36.2	1.5	2.6	46	18.9	2.0	3.5	47	19.3
	2	9.5	8.7	5.0	7.9	8	4.1	50.5	80.1	122	62.6	1.0	1.6	9	4.6	6.5	10.3	56	28.7
	3	16.1	21.9	9.5	13.9	20	10.2	51.0	75.0	97	49.2	1.5	2.2	23	11.7	6.0	8.8	57	28.9
	Ave.	22.0	26.5	12.5	19.9	30.0	14.2	44.2	70.4	102.3	48.3	1.3	2.1	26.0	12.3	4.8	7.6	53.3	25.2

TABLE 4.—(Continued)

	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
				Clean					Diseased					Clean			Diseased		
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
33. Potassium permanganate, 2% solution, dip	1	54.7	55.3	36.5	45.9	76	30.9	29.0	36.5	59	23.9	7.0	8.8	60	24.4	7.0	8.8	51	20.7
	2	9.5	6.6	7.0	9.5	15	6.6	56.5	76.9	133	58.3	0.0	0.0	0	0.0	10.0	13.6	80	35.1
	3	39.7	39.3	25.0	33.1	56	22.0	38.0	50.3	92	36.2	5.0	6.6	44	17.3	7.5	9.9	62	24.4
	Ave.	35.1	34.5	22.8	29.9	49.0	20.2	41.2	54.1	94.7	39.0	4.0	5.2	34.7	14.3	8.2	10.8	64.3	26.5
34. Potassium permanganate, 5% solution, dip	1	64.5	59.4	41.0	56.9	91	38.9	21.5	29.9	52	22.2	5.5	7.6	48	20.5	4.0	5.6	43	18.4
	2	10.4	10.2	6.5	9.7	11	5.9	52.0	77.6	111	59.0	0.5	0.7	8	4.3	8.0	11.9	58	30.9
	3	18.5	16.5	12.0	17.8	36	13.5	47.0	69.6	139	52.3	0.5	0.7	8	3.0	8.0	11.9	83	31.2
	Ave.	31.9	29.4	19.8	28.7	46.0	20.1	40.2	58.3	100.7	43.9	2.2	3.2	21.3	9.3	6.7	9.7	61.3	26.7
35. Dupont dust No. 15, 2 oz. per bu., before cutting	1	18.1	29.2	7.0	11.5	23	9.7	42.0	68.9	98	41.5	2.0	6.6	46	19.5	8.0	13.1	69	29.2
	2	8.7	11.3	5.0	8.3	11	5.9	48.0	79.7	120	65.2	0.3	0.4	10	5.4	7.0	11.6	43	23.4
	3	5.6	6.8	3.0	5.2	9	4.4	51.0	89.1	146	70.9	0.3	0.4	5	2.4	3.0	5.2	46	22.3
	Ave.	10.9	16.6	5.0	8.4	14.3	6.9	47.0	78.9	121.3	58.1	1.5	2.5	20.3	9.7	6.0	10.1	52.7	25.3
36. Check (clean seed)	1	92.7	85.7	47.5	87.2	142	58.2	3.5	6.4	18	7.4	3.0	5.5	67	27.5	0.5	0.9	17	6.9
	2	13.1	12.2	7.0	10.8	14	7.1	50.0	76.9	122	61.6	1.5	2.3	10	5.1	6.5	10.0	52	26.3
	3	65.4	67.6	37.5	59.1	99	42.9	18.0	28.3	32	13.9	4.0	6.3	57	24.7	4.0	6.3	43	18.6
	Ave.	54.9	57.8	30.7	50.3	85.0	37.9	23.8	39.0	57.3	25.5	2.8	4.6	44.7	19.9	3.7	6.1	37.3	16.6
37. Check (clean seed), mercuric chloride (1-1,000), 1½ hrs.	1	89.8	92.5	51.0	80.3	104	55.9	3.0	4.7	10	5.4	9.5	14.9	68	36.6	Tr.	Tr.	4	2.2
	2	99.6	98.6	68.0	95.4	175	79.5	0.2	0.4	2	0.9	3.0	4.2	42	19.1	Tr.	Tr.	1	0.5
	3	45.5	45.7	29.0	43.3	57	34.8	34.0	50.7	71	43.3	1.5	2.2	18	10.9	2.5	3.7	18	10.9
	Ave.	80.3	81.4	49.3	73.4	112.0	58.9	12.4	18.5	27.7	14.6	4.7	6.9	42.7	22.5	0.8	1.2	7.7	4.0
38. Check (diseased seed)	1	32.4	34.6	12.5	25.3	31	15.3	26.5	38.5	70	34.7	3.5	7.1	39	19.3	7.0	14.1	62	30.7
	2	10.8	22.2	7.0	10.1	22	11.6	57.5	82.7	106	56.1	0.5	0.7	20	10.6	4.5	6.5	41	21.7
	3	29.1	22.4	17.0	28.7	44	20.1	39.0	65.8	120	54.8	0.3	0.4	5	2.3	3.0	5.1	50	22.8
	Ave.	22.8	26.4	12.2	20.5	32.3	15.9	41.0	69.0	98.7	48.5	1.4	2.3	21.3	10.5	4.8	8.1	51.0	25.1
39. Dupont dust No. 15, 3 oz. per bu., after cutting	1	65.4	63.8	35.5	54.6	63	33.5	20.0	30.8	40	21.3	7.0	10.8	57	30.3	2.5	3.8	28	14.9
	2	22.0	31.7	12.5	17.7	30	15.6	51.0	72.3	103	53.6	3.0	4.3	31	16.1	4.0	5.7	28	14.6
	3	47.3	49.0	31.0	45.8	75	39.5	35.0	51.7	84	44.2	1.0	1.5	18	9.5	0.7	1.1	13	6.8
	Ave.	44.3	48.1	26.3	38.8	56.0	29.5	35.3	62.1	75.7	39.8	3.7	5.5	35.3	18.6	2.4	3.5	23.0	12.1
40. Formalin (1-120), 125° F., 4 min.	1	100.0	100.0	30.0	83.3	73	56.6	0.0	0.0	0	0.0	6.0	16.7	56	43.4	0.0	0.0	0	0.0
	2	92.0	89.1	57.0	91.2	132	80.0	5.0	8.0	18	10.9	0.5	0.8	15	9.1	0.0	0.0	0	0.0
	3	93.7	94.2	34.5	78.9	78	56.5	2.5	5.7	5	3.6	6.5	14.8	52	37.7	0.3	0.6	3	2.2
	Ave.	94.5	94.0	40.5	85.4	94.3	65.5	2.5	5.2	7.7	5.3	4.3	9.1	41.0	28.5	0.1	0.2	1.0	0.7

TABLE 4.—(Continued)

Treatment	Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers								Culls							
				Clean				Diseased				Clean				Diseased			
				Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%	Wt. ^a	%	No.	%
41. Special dust No. 90, 2 oz. per bu., before cutting	1	94.5	92.4	28.0	77.8	68	47.6	2.0	5.6	6	4.2	6.0	16.7	64	44.8	Tr.	5	3.5	
	2	50.0	44.7	36.5	48.7	74	38.9	36.5	48.7	75	39.5	1.0	1.3	11	5.8	1.0	1.3	15.8	
	3	53.1	55.5	25.0	52.6	74	50.7	22.0	46.3	55	37.7	0.3	0.5	7	4.8	0.3	0.5	6.8	
	Ave.	60.9	62.2	29.8	56.4	72.0	45.1	20.2	38.3	45.3	28.4	2.4	4.5	27.3	17.1	0.4	0.8	9.4	
42. Special dust No. 90, 3 oz. per bu., after cutting	1	12.1	8.6	7.5	10.7	14	7.1	57.0	81.4	132	66.7	1.0	1.4	3	1.5	4.5	6.4	24.7	
	2	79.8	82.6	43.5	76.3	84	58.3	10.0	57.5	18	12.5	3.0	5.3	35	24.3	0.5	0.9	7	
	3	84.9	81.7	46.0	84.0	120	71.0	8.0	14.6	24	14.2	0.5	0.9	18	10.7	0.3	0.5	4.1	
	Ave.	55.8	53.6	32.3	53.3	72.7	42.7	25.0	41.3	58.0	34.0	1.5	2.5	18.7	10.9	1.8	2.9	12.3	
43. Check (clean seed)	1	4.1	14.1	2.0	3.3	9	3.9	49.0	80.9	113	50.0	0.5	0.8	23	10.2	9.0	14.9	35.8	
	2	18.5	22.5	10.5	16.9	32	15.3	28.2	13.3	78.2	63.6	1.0	1.6	15	7.2	2.0	3.2	29	
	3	13.3	11.0	7.0	13.3	26	10.2	36.5	69.5	106	41.4	Tr.	Tr.	2	0.8	9.0	17.1	47.7	
	Ave.	12.0	15.5	6.5	11.1	22.3	9.7	44.7	76.5	117.3	50.9	0.5	0.9	13.3	5.8	6.7	11.5	77.3	
44. Check (clean seed), mercuric chloride (1-1,000), 1½ hrs.	1	100.0	100.0	63.5	85.8	179	66.3	0.0	0.0	0	0.0	10.5	14.2	91	33.7	0.0	0.0	0	
	2	82.3	82.1	60.0	80.3	162	68.9	13.0	17.4	38	16.2	1.5	2.0	31	13.2	0.3	0.3	4	
	3	85.6	82.3	59.0	82.8	154	64.7	10.0	14.3	34	14.3	2.0	2.8	42	17.6	0.3	0.4	8	
	Ave.	89.2	88.7	60.8	82.8	165.0	66.6	7.7	10.5	24.0	9.7	4.7	6.4	54.7	22.1	0.2	0.3	4.0	
45. Check (diseased seed)	1	79.1	81.1	38.5	57.5	130	39.6	11.5	17.2	35	10.7	14.5	21.6	136	41.5	2.5	3.7	27	
	2	16.1	11.9	10.0	15.3	18	8.6	46.0	70.2	106	50.5	0.5	0.8	7	3.3	9.0	13.7	79	
	3	14.9	21.9	6.0	12.8	26	14.6	38.0	80.9	93	52.2	1.0	2.1	13	7.3	2.0	4.2	46	
	Ave.	39.3	46.1	18.2	30.4	58.0	24.3	31.8	59.2	78	32.7	5.3	8.9	52.0	21.8	4.5	7.5	50.7	
46. Special dust No. 90, 10% solution, dip	1	13.4	20.5	9.0	12.1	25	9.7	61.0	81.9	164	63.3	1.0	1.3	28	10.8	3.5	4.7	42	
	2	68.3	74.7	47.0	67.6	126	64.9	22.0	31.7	49	25.3	0.5	0.7	19	9.8	0.0	0.0	0	
	3	38.9	46.9	27.0	36.2	71	33.0	44.0	59.1	92	42.8	2.0	2.7	30	13.9	1.5	2.0	22	
	Ave.	39.5	44.7	27.7	37.9	74.0	33.2	42.3	58.0	101.7	45.7	1.2	1.6	25.7	11.5	1.7	2.3	21.3	
47. Special dust No. 89, 2 oz. per bu., before cutting	1	3.5	2.8	2.0	2.8	3	1.2	61.5	85.4	156	62.2	0.5	0.7	4	1.6	8.0	11.1	88	
	2	22.8	16.6	16.0	21.8	32	14.0	47.5	64.8	118	51.8	0.8	1.0	6	2.6	9.0	12.3	72	
	3	12.6	16.7	7.0	12.2	27	12.9	44.0	83.8	124	59.0	0.3	0.4	8	3.8	2.0	3.5	51	
	Ave.	13.0	11.6	8.3	12.3	20.7	9.0	52.3	77.6	132.7	57.8	0.5	0.7	6.0	2.6	6.3	9.3	70.3	
48. Special dust No. 89, 3 oz. per bu., after cutting	1	14.9	22.7	9.0	13.4	21	9.9	55.0	82.1	134	63.5	1.0	1.5	27	12.8	2.0	3.0	29	
	2	60.6	55.6	38.0	57.6	86	38.6	21.0	31.8	54	24.2	2.0	3.0	38	17.0	5.0	7.6	45	
	3	47.9	51.8	32.0	45.1	84	33.2	34.0	47.9	79	31.2	2.0	2.8	47	18.6	3.0	4.2	43	
	Ave.	41.2	44.1	26.3	38.7	63.7	27.8	36.7	53.9	89.0	38.9	1.7	2.5	37.3	16.3	3.3	4.9	39.0	

TABLE 4.—(Concluded)

Plot	Total control in per cent by wt.	Total control in per cent by no.	Marketable tubers										Culls					
			Clean					Diseased					Clean			Diseased		
			Wt.*	%	No.	%	Wt.*	%	No.	%	Wt.*	%	Wt.*	%	No.	%	No.	%
1	16.2	24.6	8.0	11.3	21	9.4	51.0	71.8	104	46.6	3.5	4.9	34	15.2	8.5	11.9	64	28.7
2	0.8	3.5	0.5	0.8	6	3.0	52.0	86.7	123	61.8	Tr.	Tr.	1	0.5	7.5	12.5	69	34.7
3	10.3	17.1	5.5	8.7	15	7.8	51.5	81.1	109	56.5	1.0	1.6	18	9.3	5.5	8.7	51	26.4
Ave.	9.5	15.4	4.7	7.2	14.0	6.8	51.5	79.4	112.0	54.6	1.5	2.3	17.7	8.6	7.2	11.1	61.3	29.9
1	31.7	27.6	19.0	30.9	41	19.5	34.0	55.3	86	40.9	0.5	0.8	17	8.1	8.0	13.0	66	31.4
2	80.8	76.7	52.0	80.0	146	64.0	12.0	18.5	39	17.1	0.5	0.8	29	12.7	0.5	0.8	14	6.1
3	68.5	59.3	36.0	64.9	88	38.1	14.5	26.1	45	19.5	2.0	3.6	49	21.2	3.0	5.4	49	21.2
Ave.	60.4	55.3	35.7	58.8	91.7	41.1	20.2	35.3	56.7	25.4	1.0	1.6	31.7	14.2	3.8	6.3	43.0	19.3
1	28.7	29.6	17.0	27.9	44	24.6	40.5	66.4	88	49.2	0.5	0.8	9	5.0	3.0	4.9	38	21.2
2	92.4	91.0	59.0	85.2	137	67.8	5.0	7.2	14	6.9	5.0	7.2	47	23.2	0.3	0.4	4	1.9
3	95.6	87.8	47.0	81.7	101	51.3	2.0	3.5	5	2.5	8.0	13.9	72	36.5	0.5	0.9	19	9.6
Ave.	72.6	71.0	41.0	65.5	94.0	48.8	15.8	25.2	35.7	18.5	4.5	7.1	42.7	22.2	1.3	2.1	20.3	10.5
1	17.6	23.0	6.0	9.6	15	6.4	43.5	69.6	110	46.8	5.0	8.0	39	16.6	8.0	12.8	71	30.2
2	2.1	3.1	1.5	2.1	7	3.1	61.0	84.1	139	61.5	0.0	0.0	0	0.0	10.0	13.8	80	35.4
3	4.2	4.1	2.0	4.2	7	4.1	40.0	83.3	91	53.5	0.0	0.0	0	0.0	6.0	12.5	72	42.4
Ave.	8.0	10.8	3.2	5.2	9.7	4.6	48.2	78.9	113.3	53.9	1.7	2.3	13.0	6.2	8.0	13.1	74.3	35.3
1	44.3	44.1	11.0	31.4	33	18.2	11.5	32.9	30	16.6	4.5	12.9	47	25.9	8.0	22.9	71	39.2
2	24.7	25.7	12.0	23.8	37	17.6	36.0	71.3	120	57.1	0.5	0.9	17	8.1	2.0	3.9	36	17.1
3	14.2	10.5	7.0	13.7	21	9.2	42.0	81.9	134	58.3	0.3	0.5	3	1.3	2.0	3.9	70	30.7
Ave.	25.8	25.5	10.0	21.9	30.3	14.7	29.8	65.4	94.7	45.9	1.8	3.9	22.3	10.8	4.0	8.8	59.0	28.6
1	68.5	66.1	21.5	58.9	65	30.7	11.0	30.1	46	21.7	3.5	9.6	75	35.4	0.5	1.4	26	12.3
2	71.8	74.5	23.0	64.8	77	53.1	9.0	25.4	25	17.2	2.5	7.0	31	21.4	1.0	2.8	12	8.3
3	56.8	57.7	33.0	49.3	74	35.9	26.0	38.8	57	27.7	5.0	7.5	45	21.8	3.0	4.5	30	14.6
Ave.	63.6	65.2	25.8	55.7	72.0	38.4	15.3	33.0	42.7	22.7	3.7	7.9	50.3	26.8	1.5	3.2	22.7	12.1
1	81.6	83.2	44.0	73.3	114	49.1	10.0	16.7	28	12.1	5.0	8.3	79	34.1	1.0	1.7	11	4.7
2	70.0	68.7	26.0	52.0	66	33.8	11.5	23.0	31	15.9	9.0	18.0	68	34.9	3.5	7.0	30	15.4
3	22.2	25.4	9.5	21.1	28	13.7	31.0	68.9	96	46.8	0.5	1.1	24	11.7	4.0	8.9	57	27.8
Ave.	60.7	60.0	26.5	51.4	69.3	32.9	17.5	33.9	51.7	24.5	4.8	9.3	57.0	27.1	2.8	5.4	32.7	15.5

* Weight in lbs.

when the checks were least free; and the results in 1924 were intermediate. Furthermore, very poor control was obtained with this disinfectant in 1926.

The fact that the results obtained with mercuric chloride approximated the same curve as the results of the checks might be expected. However, it might be assumed that the only criterion in determining the amount of soil infestation is the amount of disease which develops when treated clean seed is planted. But one will note (table 5, treatment 29) that approximately 25 per cent of the potatoes grown in infested soil in 1926 were infected. If the above assumption is correct, one would have to concede that the treatment given this check was absolute in controlling any infection from the organism on the seed. If this same treatment gave absolute control of infected seed, then any lot of treated diseased seed might be expected to produce approximately the same percentage of clean product as clean seed treated in like manner. This is not the case, as one will see by glancing at treatment 12 in table 5. Theoretically, then, the difference between the percentages of the clean product of treatment 12 and treatment 29 (1926) should indicate the difference in effectiveness of the two treatments or a lack of uniformity in soil infestation. The latter is in all probability the case, for treatment 12 is the result of but one trial replicated three times, while treatment 29 is the average of eight trials, each replicated three times.

The opposite may be true for the hot formalin (treatment 8). Although this disinfectant produced the highest percentage of control in 1926, it, like treatment 12, is the result of but one trial replicated three times. There is one outstanding fact concerning hot formalin, however, which is not exhibited by the other disinfectants, and that is, that regardless of what results the treated checks produced, the results obtained with hot formalin were quite uniform for the three years' trials.

Results obtained with Dupont Dust No. 15 were not uniform. The disinfectant used in the three years' trials came from the same container. Best control was obtained with it in 1924 and poorest in 1926.

Semesan Dust was like mercuric chloride in the manner in which it followed the same curve as the checks. Excellent control was obtained with it in 1925, but the results obtained in 1926 were not good enough to warrant its use. It is evident, therefore, that more tests will have to be conducted before any definite recommendations can be made in respect to these proprietary disinfectants.

In conclusion the authors wish to explain their reason for reading the results from the standpoint of sclerotia upon the surfaces of the tubers, and not from the standpoint of stem lesions. In the Palouse district in the vicinity of Moscow, Idaho, very few stem lesions ordinarily develop. Very seldom is a poor stand due to *Corticium vagum* var. *solani* Burt., and rarely can aerial tubers, due to the same cause, be found. The authors do not

TABLE 5.—*Comparison of various disinfectants used for more than one year in controlling rhizoctonia of potatoes at Moscow, Idaho*

Treatment	1924		1925		1926	
	Clean tubers in per cent by		Clean tubers in per cent by		Clean tubers in per cent by	
	wt.	no.	wt.	no.	wt.	no.
1. Check, diseased seed, no treatment.....	31.7 ^a	30.3 ^a	46.8 ^a	51.4 ^a	24.7 ^b	27.0 ^b
2. Formalin (1-240), 1½ hrs. soak.....	23.6	23.3	83.4	84.3		
3. do, presprinkled.....	40.2	41.3	84.4	86.6		
4. Formalin (1-120), 50° C., 4 min.	78.9	78.7	74.3	75.5		
5. do, presprinkled.....	82.7	80.5	81.3	84.0		
6. Formalin (1-120), 55° C., 4 min.	71.6	73.9	84.3	83.4		
7. do, presprinkled.....	87.6	86.8	86.2	87.9		
8. Formalin (1-120), presprinkled, 125° F., 4 min.					94.5	94.0
9. Furfural (1-60), 50° C., 10 min.	82.3	76.4	89.3	89.0		
10. do, presprinkled.....	79.6	78.3	88.7	90.4		
11. Mercuric chloride, (1-1,000), 1½ hrs. soak	74.9	76.3	92.8	91.8		
12. do, presprinkled.....	88.8	88.0	92.9	93.5	50.4	49.6
13. Dupont dust No. 15, 3 oz. per bu., before cutting	57.8	59.8	55.5	58.8		
14. do, presprinkled.....	90.3	90.3	65.7	66.3		
15. Dupont dust No. 15, presprinkled, 2 oz. per bu., before cutting			57.0	59.4	10.9	16.6
16. Dupont dust No. 15, presprinkled, 3 oz. per bu., after cutting					44.3	48.1
17. Dupont dust No. 12, 0.1666% solu- tion, 1½ hrs. soak	42.5	46.3				
18. do, presprinkled.....	32.9	34.4				
19. Dupont dust No. 12, presprinkled, 2 oz. per bu., before cutting					8.8	12.6
20. Dupont dust No. 12, presprinkled, 3 oz. per bu., after cutting					19.0	23.6
21. Dupont dust No. 12, presprinkled, 10% solution, dip					12.9	13.0
22. Semesan dust, 3 oz. per bu., before cutting	68.7	69.2	95.4	96.2		
23. do, presprinkled.....	81.4	80.9	96.1	96.2		
24. Semesan dust, presprinkled, 2 oz. per bu., before cutting			98.5	98.3	45.9	47.4

TABLE 5.—(Continued)

Treatment	1924		1925		1926	
	Clean tubers in per cent by		Clean tubers in per cent by		Clean tubers in per cent by	
	wt.	no.	wt.	no.	wt.	no.
25. Semesan dust, presprinkled, 3 oz. per bu., after cutting					72.7	74.3
26. Semesan dust, 0.125% solution, 1½ hrs. soak	56.5	58.0				
27. do, presprinkled	60.6	60.4				
28. Check (clean seed)	67.3	70.9	88.8	91.1	57.7 ^b	55.9 ^b
29. Check (clean seed), mercuric chloride, presprinkled (1-1,000), 1½ hrs. soak	74.0	72.9	91.6	91.4	75.4 ^b	73.8 ^b
30. Bayer dust, presprinkled, 2 oz. per bu., before cutting			83.9	84.3	29.9	39.0
31. Bayer dust, presprinkled, 3 oz. per bu., before cutting			85.5	86.4		
32. do, after cutting					53.1	56.6

^a Average of two checks.

^b Average of eight checks.

claim that such symptoms do not occur, but that when they do they are the exception and not the general rule. We offer the following data in explanation of this. The average rainfall in inches during the growing months for the past 35 years is as follows:

May	June	July	August	September	October
2.16	1.37	0.64	0.79	1.22	1.61

The average temperatures in degrees Fahrenheit for the same period are as follows:

May	June	July	August	September	October
52.0	58.6	66.6	66.2	57.4	48.3

The plots are planted about June 1 of each year, and harvested between October 1 and 15. Thus the potatoes miss the heaviest rainfall and lowest temperatures in the spring, during the time of germination, and are harvested before the heaviest fall rains occur.

SUMMARY

Potato seed treatment experiments for the control of rhizoctonia similar to those conducted in 1924 and in previous years were again conducted in

1925 and 1926. Various proprietary compounds were used in comparison with the cold corrosive sublimate, and the cold and hot formalin methods. In 1925 there were 31 different treatments, including the checks, best control being obtained with Semesan Dust when applied at the rate of two ounces per bushel to presprinkled seed. In 1926, there were 55 various treatments including the checks, best control being obtained when presprinkled potatoes were dipped in a 1-120 formalin solution, at 125° F., for four minutes.

Because the results obtained with Dupont Dust No. 15 were so satisfactory in 1924, a series of tests with this compound was conducted in 1925 in addition to the regular tests. The results obtained in 1925 did not compare in any way with the results obtained with the same compound in 1924.

In all of the tests considerable emphasis was placed on sprinkling the potatoes before treatment.

Sclerotia on the tuber surface at harvest time were the basis of disease readings rather than stem lesions during the growing season.

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THE NATURE OF RESISTANCE IN ZEA MAYS L. TO PUCCINIA
SORGHI SCHW.

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INTRODUCTION

The study of disease-resistance in plants is one of the most fascinating phytopathological problems, both from a theoretical and from a practical viewpoint. It naturally divides itself into three parts: (1) the mere demonstration of resistance, (2) the genetical nature of resistance, (3) the morphological or physiological nature of resistance.

The literature on the nature of resistance was reviewed by Butler (5) in 1918; by Howitt (9) in 1923-1924, who gives an extensive bibliography; and by Walker (22) in 1924. Walker (22, p. 241) concludes: “. . . the true nature of resistance has been exceedingly difficult to determine and, moreover, it is quite obvious that almost every case of resistance very probably presents a specific problem.”

The present paper deals with the nature of corn rust resistance. Several previous workers studied the nature of resistance to rust fungi in other cereals (1, 2, 3, 4, 7, 8, 10, 14, 17, 18, 24, and 25). Their results will be compared with the writer's results later.

MATERIALS AND METHODS

The work was started with two of the physiologic forms of *Puccinia sorghi*,² as mentioned by Stakman and Christensen (19). In order to find lines of corn suitable for a study of the nature of resistance, a number of selfed lines were studied with respect to their rust reaction when inoculated artificially in the greenhouse. Two lines were selected for further study. If we call the corn lines A and B, and the rust forms 1 and 2, the rust reaction, expressed in symbols of Stakman and Levine's scale for *P. graminis tritici* (20), was as follows:

	Form 1	Form 2
A	4	1
B	1	4

¹ The work described in this paper was done while the writer was a fellow of the International Education Board, to which he wishes to express his great indebtedness for the opportunities given to him to carry on the present research. Many thanks are due to Dr. E. C. Stakman, head of the Section of Plant Pathology of the University of Minnesota, St. Paul, upon whose suggestion the investigations were started, for hospitality in his laboratory, and for much valuable help.

² It has been impossible to compare these two forms of *Puccinia sorghi* with those studied by Mains (13).

Attempts were made to picture the course of infection in both lines of corn. Both forms of rust were originally used, but form 2 proved to be more virulent in susceptible hosts and gave better results. The results described in this paper refer to form 2.

A number of corn plants were inoculated in the usual way (21, p. 432), and daily fixations, during at least 12 successive days, were made of inoculated plants. Controls were made of the type of reaction of the individual plants by fixing only a part of the inoculated leaves or by re-inoculating newly developed leaves. By fixing, cutting, and examining at least four leaves every day, the typical stages of development of the fungus in the leaf were determined.

Most of the material was fixed in chrom-acetic acid (6, p. 25). Gilson's fluid (6, p. 225) was unsuccessful in that it did not fix the host tissue so well as it fixed the fungus. In order to promote penetration of the fixing fluid, the material was placed in vacuum for five minutes. This, however, caused a loss of some of the inoculum in the early stages. Perfect results were obtained by immersing the whole inoculated leaf in the fixing fluid for three or four hours and cutting it into small pieces afterwards.

The sections were cut 8 or 10 μ thick and were stained with saffranin, gentian violet, and organge G, according to the following time schedule:

Saffranin, 1 per cent in 50 per cent alcohol; 5 minutes

Dip in water

Gentian violet, 1 per cent in water; 10 minutes

Dip in water, in alcohol 70 per cent, in alcohol 95 per cent

Few dips in 1 per cent orange G in clove oil

Few dips in clove oil

Xylol, a few minutes

Mount in balsam.

EXPERIMENTAL RESULTS

The Course of Infection in the Susceptible Corn-line

An infected leaf of the susceptible line is shown in figure 1, A. The fungus forms rather large uredo-sori which are not isolated but become confluent. No necrotic or chlorotic areas develop. Microscopically, the infection takes place as follows: The germ tube grows toward a stoma, whereupon it usually forms an appressorium. A very thin infection thread pushes its way through the stomatal slit and becomes a hypha which immediately continues to grow toward some neighboring host cells. The second day after inoculation, the typical stage of development is the one shown in Plate XXX, A. In quite a few cases, however, the mycelium has already penetrated intercellularly in the parenchymatous leaf tissue (Pl. XXX, B). The mycelium

develops further and on the fourth day after inoculation reaches the stage shown in Plate XXX, C. During the following days the whole leaf is invaded by the fungus, which continues its intercellular growth without changing its form. When a certain portion of a leaf has been invaded, the mycelial threads beneath the epidermis thicken considerably. This stage, shown in Plate XXX, D, is usually reached the seventh day after inoculation and is an introduction to spore formation. The next day (Pl. XXX, E) young spores are present, in which the binucleate condition is usually very clear. Plate XXX, F shows all stages of development, *i.e.*, penetration at the right, mycelial development in the parenchyma, and ripe spores at the left. Spores are formed in large quantities, as shown in Plate XXX, G. The newly

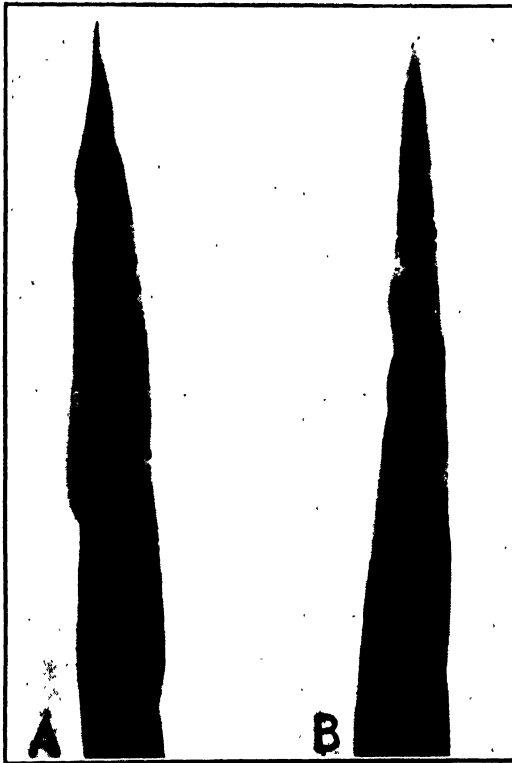


FIG. 1.—Types of infection produced by *Puccinia sorghi* form 2 on seedlings of *Zea mays*:
A. On a susceptible line of corn; B. On a resistant line.

formed spores are liberated, and evidently no more than one set of spores is formed in one sorus. Plate XXX, H shows an almost empty sorus on the eleventh day after inoculation; it is remarkable that there is not the least indication of a general necrosis of host tissue at this stage of development and, as a fact, necrosis has not been found at all in the susceptible host.

Two main features in the course of infection of the susceptible host are:

1. There is no evidence of any difficulty which the fungus meets in its development on account of the host plant.
2. The host tissue does not show any sign of suffering from the fungus.

The Course of Infection in the Resistant Corn-leaf

Figure 1, B shows the disease symptoms as developed in the resistant host: small isolated uredo sori, surrounded by a border of necrotic tissue.

The spore germination, formation of appressoria, and penetration are apparently the same as in the susceptible host. On the second day after inoculation penetration has taken place and an almost spherical substomatal vesicle is formed (Pl. XXXI, A). This substomatal vesicle is very characteristic of the fungus in a resistant host. After this stage has been reached, the fungus stops growing for a time, so that on the fourth day after inoculation it appears unchanged (Pl. XXXI, B). At that stage, however, some hyphal outgrowths develop from the substomatal vesicle, one of which usually reaches a parenchymatous host cell and develops further (Pl. XXXI, C). This development is originally rather slow, so that on the sixth day after inoculation a stage of development is reached (Pl. XXXI, D) which corresponds to the stage on the fourth day after inoculation of a susceptible host (Pl. XXX, C). Although development is relatively slow, a whole portion of a leaf from epidermis to epidermis is invaded and on the ninth day after inoculation young spores have been formed and a few ripe spores are liberated (Pl. XXXI, E). Before abundant spore formation can take place, as occurs in the susceptible host, there is a general necrosis of both host tissue and fungus on the tenth day after inoculation (Pl. XXXI, F and G). This necrosis covers the whole infected leaf area and, of course, stops the development of the fungus.

Two main features in the course of infection of the resistant host are:

1. The development of the fungus is not continuous, as, after penetration has taken place, it stops growing for about two days.
2. After invasion of the leaf has taken place and spore formation has begun, there is a general necrosis of fungus and host tissue.

Comparison Between the Course of Infection in a Susceptible and in a Resistant Host

There are some striking differences between the behavior of the fungus in a susceptible and in a resistant host. The almost spherical shape of the substomatal vesicle is typical of the fungus in a resistant host and is probably a rest form, inasmuch as the parasite remains in this stage for about two days. There may be a distinct substomatal vesicle in a susceptible host;

never, however, is this structure a rest organ. The continuity of infection is typical of the fungus in a susceptible host. The infection thread usually gives rise to a hypha which grows without a rest period and invades the host tissue.

The fungus in a resistant host develops similarly to the fungus in a susceptible one, but later and to a lesser degree. It already has been pointed out that the development on the sixth day in a resistant host corresponds to the stage reached by the parasite in a susceptible host on the fourth day. Furthermore, the ninth day in a resistant host corresponds to the eighth day in a susceptible host. At this stage the mycelium in a susceptible host is considerably thicker than in a resistant host (Plate XXX, E and Pl. XXXI, E).

The most striking difference is the general necrosis which takes place in the resistant host just after the beginning of spore formation, but which is never found in the susceptible host.

THE NATURE OF RESISTANCE

The interpretation of the differences between infection in a susceptible host and in a resistant one is an attempt to indicate the nature of resistance. We might just as well speak of the nature of susceptibility, but, as resistance is usually the more exceptional condition, it draws more attention. Also, it is impossible, of course, to indicate the nature of resistance without, at the same time, indicating the nature of susceptibility.

In view of the fact that the two lines of corn used in the experiments react in an opposite manner to physiologic form 1 as they do to form 2, morphological or anatomical characters cannot be the basis of resistance. We deal, therefore, with a case of true physiological resistance.

It is very clear that there is a quantitative difference between the development of the fungus in a susceptible host and its development in a resistant one, both with respect to time and to fungous material. The development in a resistant host is much slower. In a susceptible host the first stages after penetration has taken place suggest that a certain substance in the host attracts the fungus, so that it grows on immediately. If this substance is not present in sufficient quantity in the resistant host, this would explain why the parasite assumes a rest form after having penetrated the stomatal slit.

The substance in question, if actually present in a larger quantity in the susceptible host than in the resistant one, is very probably a nutritive substance. The difference in quantity explains the quantitative difference between the development of the fungus in a susceptible and in a resistant host.

We have now to account for the necrosis in the resistant host, and this is not easy, for there is no indication at all as to whether the fungus or the

host cells die first. It is possible that, although the fungus in a resistant host finds enough nutritive substance to grow for a time and even to form spores, a condition arises in which all of the available limiting nutritive substance has been used; the fungus dies from starvation, and excretes a certain substance which is harmful to the host cells and causes a necrosis of them. This is a simpler explanation than the hypothesis that toxins and anti-toxins are working, because it is in harmony with the observed quantitative difference in development of the fungus in a susceptible and resistant host. Furthermore, according to the recent research of Sardiña (16), the existence of toxins and anti-toxins in plants has never been proved.

Consequently the differences between the development of the parasite in a susceptible and in a resistant host may be explained by the hypothesis that susceptibility is determined by the presence of a relatively large quantity of a certain nutritive substance which acts chemotropically on the fungus after penetration and makes possible an abundant development of mycelium and spores. This substance is present in very small quantity in the resistant host; therefore there is only a slow and meager development of the parasite. A few spores are formed, but then all the available limiting substance has been used and the fungus dies from starvation, which leads to a necrosis of the host tissue.

There are some more facts which deserve consideration in regard to the above set hypothesis. A great number of types of infection between the extremely susceptible and the extremely resistant ones occur, and there are no sharp distinctions between them. The assumption of quantitative differences among the hosts, controlling the degree of susceptibility, is a plausible explanation. In many of the studies concerning the genetic nature of disease resistance, which several investigators made, the action of multiple factors³ was found. Although nothing is known about the genetic basis of corn rust resistance, the action of multiple genetic factors would be in perfect harmony with the assumption of a quantitative nature of resistance.

The question arises whether immunity is fundamentally different from resistance. Among the writer's investigated lines of corn, one was perfectly immune from the parasite in question, showing small necrotic flecks without any pustules. Unfortunately, as a very limited quantity of seed of this line was available, only a preliminary investigation could be made. Evidently immunity was nothing but extreme resistance, as the fungus developed in the leaf without any sign of difficulty. However, necrosis started before spore formation had begun, hence at an earlier stage than in the resistant line. This too may be explained on the basis of starvation.

A consideration of the relations between the two lines of corn and the two physiologic forms of the parasite used in the experiments suggests link-

³ This term refers to the European term "polymeric."

ing up the nature of resistance with the nature of physiologic specialization. The one line of corn was resistant to form 1, and susceptible to form 2 of the rust; while the other line reacted in an opposite way. If physiologic specialization is the requirement of definite food substances and if susceptibility and resistance are determined by the presence or relative absence of these substances, the relations are explained. If this is true, physiologic specialization and resistance are two expressions of one and the same thing, namely, the relation between host and parasite.

The questions discussed may be finally settled by the demonstration of the nature of the substance controlling susceptibility and, by its relative absence, resistance. Mains (12) has shown that the development of *Puccinia sorghi* depends to a great extent on the presence of carbohydrates. It might be that the substance in question is one or another of the carbohydrates, but there is no experimental evidence whatever.

Summarizing, it may be said that there is experimental evidence that the difference between susceptibility and resistance is of a quantitative nature. This suggests that the quantity in which a special nutritive substance is present determines whether the host is susceptible or resistant. It is then plausible to account for the necrosis in resistant hosts by assuming that the fungus dies from starvation and afterwards excretes a substance which kills the host cells. The existence of all possible gradations in the degree of susceptibility between resistance and complete susceptibility is in favor of the idea of a quantitative nature of the differences in question. There is some experimental evidence that immunity is nothing but extreme resistance. Evidently physiologic specialization and resistance are two expressions of the host-parasite relation.

DISCUSSION

Investigations, similar to the one described above, with other rust fungi are reported by several authors. Marshall Ward (24, 25) was the first to study the host-parasite relation in *Bromus* species with *Puccinia dispersa* and in *Triticum sativum* with *P. glumarum*. He originally was inclined to ascribe the nature of resistance to the action of toxins or anti-toxins. When he failed to isolate or to demonstrate the existence of such bodies, he was in doubt whether resistance was due to the interaction of toxins and anti-toxins or to a starvation process.

Gibson (7), working in Ward's laboratory, studied the infection of several rust fungi, especially of *Uredo chrysanthemi* in a number of different host plants. She rejects the idea of starvation. Dorothea Marryat (14), also working in Ward's laboratory, favors the toxin-anti-toxin idea in the case of *Puccinia glumarum* on wheat. She writes (14, p. 137): "We are therefore forced to fall back upon the theory that immunity to disease is

due in these cases to the production of certain toxins and anti-toxins by host or parasite, or both, which are mutually destructive." It is not evident in Miss Marryat's work which of the two dies first, host or parasite. This is very important, because, when the fungus dies first, starvation may be the cause just as well as a toxic action.

Stakman (18, p. 197), relating to the immunity against *Puccinia graminis*, writes: "Hyphae at the end of five days from the time of inoculation have sometimes killed most of the cells in their immediate vicinity, but still remain alive, although they are usually not vigorous." This would be in favor of a toxic action. In another study, however (17, p. 46), he finds: ". . . they"—the tips of hyphae—"may disintegrate without having first killed host cells." His final conclusion is in favor of the toxin-anti-toxin idea.

Extensive histological studies on the course of infection of *P. graminis* and *P. tritici* were made by Ruth Allen (1, 2, 3, 4). In so far as her work has some bearing on the questions discussed now, she favors the idea that antagonistic reactions between host and parasite form the nature of immunity (1, p. 148). Observations of Helen Hart (8, p. 192) on the infection of *Melampsora lini* in immune flax pointed out that ". . . some of the host cells are killed by the fungus, which is unable to establish itself and finally dies." This would be in favor of an antagonistic action.

The only observations on corn rust reported in the literature are those of Weber (23) and of Mabel Agnes Rice (15, pp. 102-122), who studied especially the development of haustoria. These studies, however, do not give any clue to the nature of resistance.

Considering the different observations reviewed above, there appears to be a general tendency to accept the theory that toxic and anti-toxic reactions determine resistance and immunity, and in several cases there is good evidence. The writer's work, however, gives strong support to the theory that, in corn, resistance to *P. sorghi* is the expression of starvation of the parasite. Many of the results favor the starvation hypothesis of Leach (10, p. 85) on the nature of resistance to *P. graminis*, for which hypothesis there was "very little original experimental evidence" (10, p. 86) when offered. The same author (11) favors the theory of starvation in the case of resistance in beans to *Colletotrichum lindemuthianum*.

It is evident, therefore, that corn rust resistance is of a different nature than resistance to some other rust fungi.

SUMMARY

1. Inoculations with two physiologic forms of *Puccinia sorghi* Schw. on a large number of selfed lines of corn showed that a certain line was sus-

ceptible to one rust form and resistant to the other, while another line reacted in an opposite way.

2. A histological study was made of the course of infection of one of the forms of the parasite on the two corn lines mentioned above.
3. The following differences between the infection of the susceptible and of the resistant host were detected:
 - a. After penetration, the parasite in the resistant host forms an almost spherical substomatal vesicle and stops growing for about two days; in the susceptible host the fungus grows on immediately after penetration.
 - b. The development of the fungus in the resistant host is much slower and does not progress so far as in the susceptible host.
 - c. In the susceptible host there is abundant spore-formation and no necrosis; whereas in the resistant host only a few spores are formed, and a general necrosis of both host cells and fungus follows immediately.
4. The differences are quantitative and may be explained by the assumption that the amount in which a special nutritive substance is present determines the degree of susceptibility.
5. The necrosis in the resistant host may be accounted for by the assumption that, after a few spores have been formed, the fungus dies from starvation and then excretes a substance which kills the host cells.
6. There is some experimental evidence that immunity is nothing but extreme resistance.
7. Physiologic specialization and resistance can be considered as the two expressions of the host-parasite relation.
8. Most of the investigators who studied the nature of resistance to several rust fungi other than *P. sorghi* are inclined to assume the action of toxins and anti-toxins. Since there is no such indication in the writer's work, the nature of corn rust resistance seems to be different from that in other rust fungi so far studied.

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EXPLANATION OF PLATES

PLATE XXX

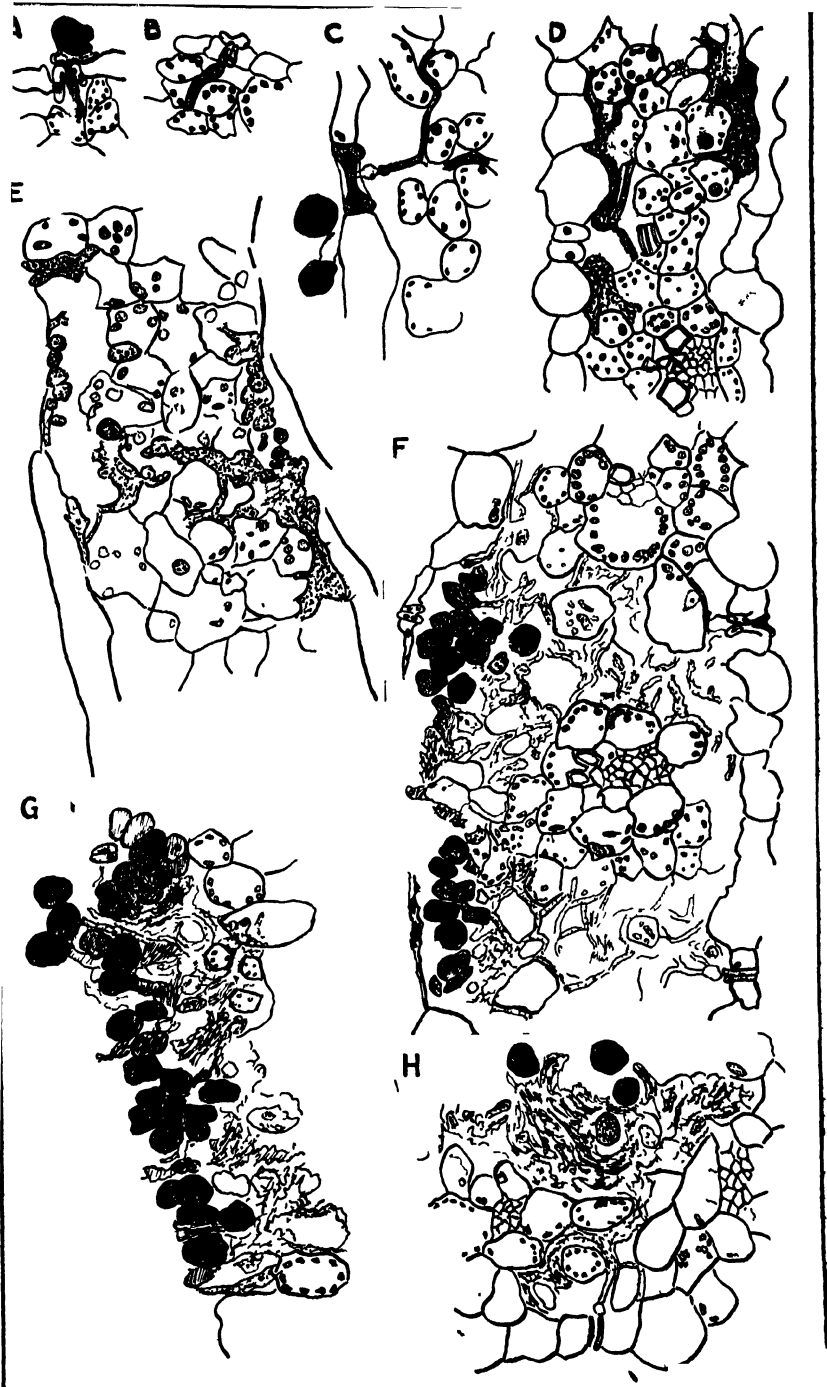
Sections of a susceptible line of corn after inoculation with physiologic form 2 of *Puccinia sorghi*, illustrating the course of infection. (Drawings made with the aid of a Leitz drawing ocular at a magnitude of about 450 and presented here at a magnitude of about 270.)

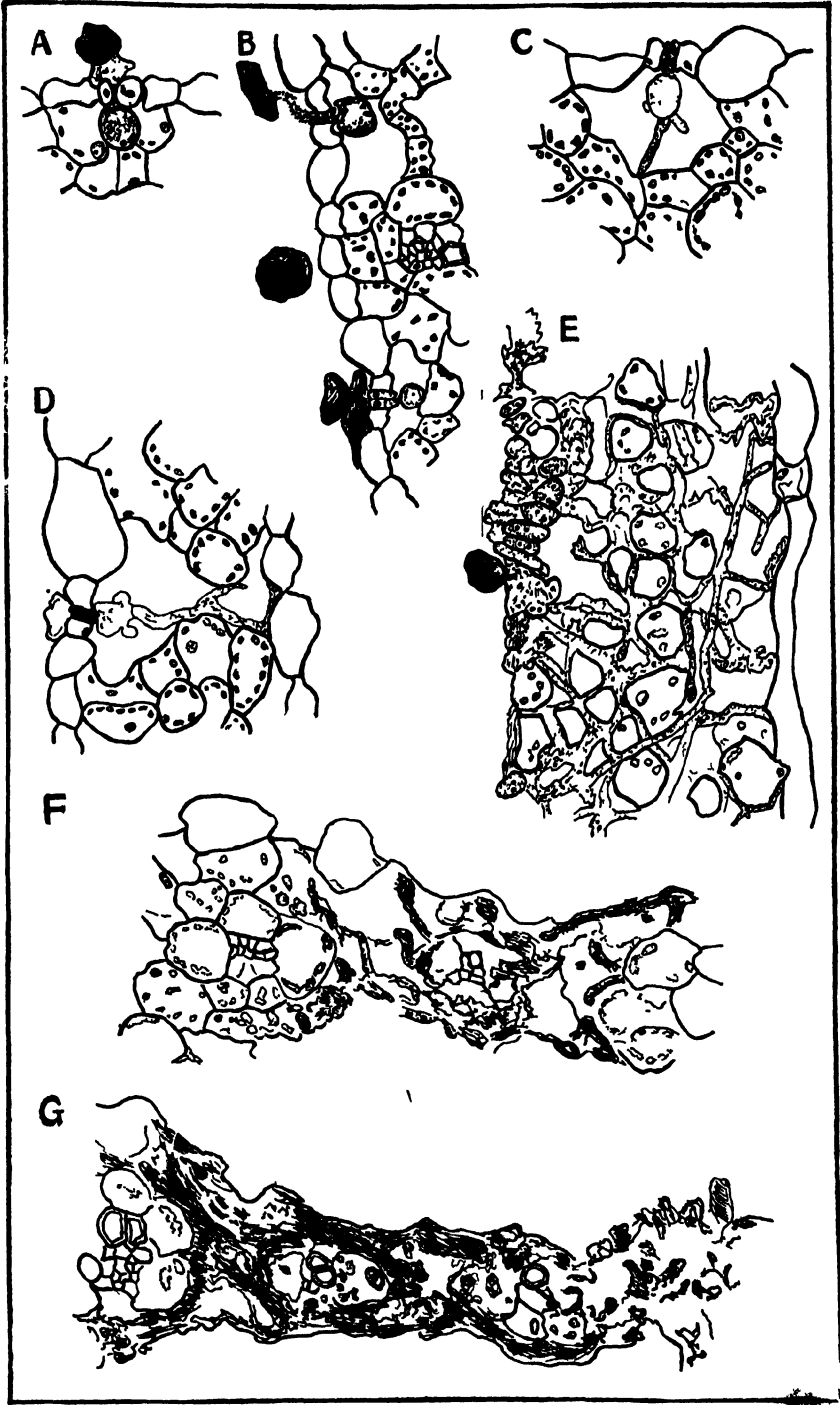
A. Two days after inoculation, showing urediniospore, appressorium, infection thread, and hypha. B. Two days—showing appressorium and hypha. C. Four days after inoculation—spores, substomatal vesicle, hypha; immediately ahead of the substomatal vesicle are two hyphal nuclei. D. Seven days—thick mycelium beneath epidermis, a preliminary to urediniospore formation. E. Eight days—young spores, many binucleate. F. Nine days—appressorium and empty substomatal vesicle (at the right); mycelium inside the leaf and a group of mature urediniospores (at the left). G. Nine days—ripe urediniospores in abundance. H. Almost empty uredo-sorus; no general necrosis of host tissues.

PLATE XXXI

Sections of a resistant line of corn after inoculation with physiologic form 2 of *Puccinia sorghi*, illustrating the course of infection. (Drawings made the same way as in Plate XXX, presented here at a magnitude of about 340.)

A. Two days after inoculation—urediniospore, appressorium, enlarged spherical substomatal vesicle. B. Four days after inoculation—same as A. C. Four days—appearance of hyphal outgrowths from enlarged substomatal vesicle. D. Six days—appressorium, substomatal vesicle, hypha. E. Nine days—mycelium inside the leaf; young, binucleate, urediniospores at the left. F. Ten days—general necrosis of host tissue and of rust fungus. G. Same as F.





HETEROTHALLISM IN *USTILAGO ZEAE*¹

E. C. STAKMAN AND J. J. CHRISTENSEN

INTRODUCTION

It has been shown by Kniep (5 and 6), Zillig (13), Bauch (2 and 3) and others that there are sexual strains in many species of smut fungi. These strains may be morphologically isogamous but functionally heterogamous. Kneip (6) has shown that fusions also may occur between different species, and he describes cases in which sporidia or germ tubes of several different species all fuse together. This is very suggestive of the origin and stability of physiologic forms in smut fungi.

Kniep (5) in 1919 showed that sporidial cultures of *Ustilago violacea* (Pers.) Fuckel. from different host plants differed in appearance. Zillig (13) in 1921 demonstrated clearly that there are distinct physiologic forms of this fungus which can be distinguished from each other by their pathogenicity for certain members of the Caryophyllaceae. Liro (7) and Bauch (4) confirmed Zillig's observations and extended them somewhat. Zillig also showed that there are sexual strains within individual physiologic forms and further that fusions may occur between sexual strains of different physiologic forms. When plants were inoculated with the sporidia of one sex only, that is, haploid sporidia, no infection resulted, indicating that the diploid condition is necessary for infection.

Stakman and Christensen (12) and Christensen and Stakman (1) demonstrated that there are many physiologic forms of *Ustilago zeae* (Beckm.) Ung., and evidence was presented that new forms might arise through mutation. It seemed quite likely that they might arise also as a result of hybridization, because of the fact that many physiologic forms or cultural types often were isolated from single collections or even from single smut galls. The large number of types thus obtained suggested very strongly that the material was heterozygous. For this reason it was decided to make a study of the possible rôle of hybridization and subsequent segregation in the origin of physiologic forms. In the meantime, however, evidence of the existence of sexual strains was obtained as a result of field experiments.

FIELD EXPERIMENTS

During the summer of 1927, an attempt was made to determine the effect of inoculating certain varieties of corn with single physiologic forms of

¹ Published with the approval of the Director as paper No. 741 of the Journal series of the Minnesota Agricultural Experiment Station.

Ustilago zeae and with combinations of two, four and eight forms. It was observed that more smut galls always developed on plants inoculated with mixed cultures than on those inoculated with a single form. However the results of inoculating with two forms sometimes were almost as good as those obtained by inoculating with four or with eight forms. It was quite evident that the virulence of mixtures of several forms was not due simply to the additive effect of the component forms. Apparently something strikingly new had happened. For this reason a field experiment was made in order to determine more accurately the results of inoculating corn with single forms and with various combinations of forms.

All of the physiologic forms used in the field experiment had been isolated from material collected in Minnesota. While they were not monosporous cultures, they had been grown from one to three years on many different media and appeared to be pure types, although sectors appeared in some of them. The forms differed from each other consistently in appearance when grown on artificial media. Because of the distinct and consistent differences in appearance and in certain physiological characters, they naturally were considered as different physiologic forms. These particular forms, designated as M2, M3, M4, and M7, were selected because they failed to cause infection when used in previous field experiments in which many other forms alone caused various degrees of infection on at least some pure lines of corn.

Inoculum for the experiments was prepared by growing each form separately in a solution of 2 per cent dextrose and 1 per cent malt-syrup. The cultures were allowed to develop for from 2 to 3 weeks before inoculations were made. They were then strained through cheese-cloth in order to remove the largest clumps of solid material. When inoculations were made with 2 forms together, the cultures were mixed just before inoculation. The inoculum was injected into the plants by means of a hypodermic syringe, as near the growing point as possible. The controls were inoculated in the same manner with sterile nutrient solution.

Between 50 and 65 plants of Golden Bantam corn were inoculated with forms M2, M3, M4, and M7 separately, and about the same number with each of the following combinations: M2 and M3, M2 and M7, M3 and M4, and M3 and M7. Golden Bantam was selected instead of a selfed line because it was known to be susceptible to a large number of physiologic forms. The results are given in table 1. Apparently no one of the forms alone caused the formation of smut galls, although it appeared as though the plants became infected, because the tissues, sometimes extending several inches from the point of inoculation, were more or less translucent, and because the plants inoculated with some forms were decidedly stunted. While galls appeared on some of the plants, they evidently resulted from

TABLE 1.—*Results of inoculating Golden Bantam corn in the field with individual physiologic forms of Ustilago zeae and with various combinations of forms*

Forms of <i>Ustilago zeae</i>	No. plants inoculated ^a	No. plants free from infection ^b	No. plants infected	
			Incipient	With galls
M2	61	61	0	0
M3	65	63	0	2
M4	59	51	0	8
M7	60	53	1	6
M ² and M3	62	2	9	51
M ² and M7	60	9	4	47
M ³ and M4	62	59	1	2
M3 and M7	51	50	1	0
Control	61	53	3	5

^a Plants from 6 to 10 inches tall when inoculated.^b When final notes were taken.

natural inoculation and, except in one case, no more galls developed on those plants inoculated with single forms than on the controls. The experiment was made rather late in the season when smut was prevalent in the field, and a certain amount of natural inoculation was to be expected. Smut galls developed on a large percentage of plants inoculated with forms M2 and M3 and with forms M2 and M7, but not on those inoculated with forms M3 and M4 and forms M3 and M7. This indicates, therefore, that there prob-

TABLE 2.—*Results of inoculating Golden Bantam corn in the greenhouse with individual physiologic forms of Ustilago zeae and with various combinations of forms*

Forms of <i>Ustilago zeae</i>	No. plants inoculated ^a	No. plants free from infection ^b	No. plants killed ^c	No. plants with galls
M2	44	44	0	0
M3	36	36	0	0
M4	40	40	0	0
M7	43	42	0	1
M2 and M3	37	12	1	24
M2 and M4	39	17	1	22
M2 and M7	43	8	19	15
M3 and M4	42	42	0	0
M3 and M7	38	38	0	0
M2, M3, M4, and M7.....	35	15	6	17
Control	44	44	0	0

^a Plants from 3 to 5 inches tall when inoculated.^b No visible evidence of infection when final notes were taken.^c Some plants were killed before galls were completely formed.

ably are at least two sexes, form M2 being of one sex, and forms M3, M4, and M7 of another.

GREENHOUSE EXPERIMENTS

Although the results of this field experiment seemed quite conclusive, the experiment was repeated in the greenhouse. The methods were essentially the same as those used in the field. The results are given in table 2.

It will be seen from table 2 that the results of the experiments in the greenhouse confirmed those obtained in the field. Smut galls were produced on a large percentage of plants inoculated with the following combinations of forms: M2 and M3; M2 and M4; M2 and M7; and M2, M3, M4, and M7, but only one was produced on any of the plants inoculated with

TABLE 3.—*Results of inoculating Golden Bantam corn in the greenhouse with individual physiologic forms of Ustilago zeae and with various combinations of forms*

Forms of <i>Ustilago zeae</i>	No. plants inoculated ^b	No. plants free from infection ^c	No. plants killed ^d	No. plants with galls
N. H. ^a	21	21	0	0
Miss.	17	17	0	0
Can.	28	28	0	0
M2	19	19	0	0
M3	16	16	0	0
M9	17	17	0	0
M2 and M3	24	2	11	14
M2 and M4	18	1	8	14
M2 and Miss.	23	1	12	16
M3 and Miss.	25	25	0	0
M2 and Can.	21	3	12	10
M3 and Can.	20	20	0	0
M2 and N. H.	21	1	1	19
M3 and N. H.	24	24	0	0
M2 and M9	22	1	1	20
M3 and M9	24	24	0	0
Miss. and Can.	23	23	0	0
N. H. and Miss.	24	24	0	0
Can. and N. H.	22	22	0	0
M9 and Miss.	29	29	0	0
Control	23	23	0	0

^a N. H. refers to the form from New Hampshire; Miss. the form from Mississippi; and Can. the form from Canada.

^b The plants were from 6 to 10 inches tall when inoculated.

^c No visible evidence of infection when final notes were taken.

^d Some plants were killed before galls were completely formed.

single forms. It is evident from these experiments that form M2 is of one sex and M3, M4, and M7 are of opposite sex.

Still another experiment was made in the greenhouse. Plants were inoculated with various combinations of forms, as indicated in table 3. In table 4 are summarized all the pairings which were made. Apparently M2 is of one sex while all the other forms used are of the opposite sex.

TABLE 4.—Summary of results of inoculating Golden Bantam corn with various physiologic forms of *Ustilago zeae*

Form	N. H.	Miss.	Can.	M2	M3	M4	M7	M9
N. H.	- ^a	-	-	+	-			
Miss.	-	-	-	+	-			-
C. L.	-	-	-	+	-			
M2	+	+	+	-	+	+	+	+
M3	-	-	-	+	-	-	-	-
M4				+	-	-		
M7				+	-		-	
M9		-		+	-			-

^a -, no galls formed; +, galls formed.

OBSERVATIONS ON SEXUAL FUSIONS

The question naturally arises as to where the sexual fusions occur. It is well known that the sporidia of some of the smut fungi copulate, but there is conflicting evidence on this point regarding the behavior of *Ustilago zeae*. Maire (8) and Sartoris (10) both state that the sporidia fuse, and the former shows a figure of fused sporidia. However, Seyfert (11) questions whether the sporidia actually do fuse and states that the methods used so successfully to demonstrate the fusion of sporidia in many other smut fungi have not been successful in the case of *U. zeae*. According to Rawitscher (9) and Seyfert (11) the sexual fusions of *U. zeae* occur in the corn plant before the formation of chlamydospores. According to Rawitscher the process is as follows. The sporidia are uninucleate. They may cause infection of the corn plant, in which they produce an intercellular uninucleate mycelium. The mycelium breaks up into short hyphae which are still uninucleate. Neighboring cells may fuse, thus giving rise to binucleate cells which eventually become chlamydospores. Rawitscher

was not able to observe the actual process of nuclear fusion because of the small size of the nuclei. However, he states that it must occur in the rather young spore cells, as the older ones and the mature chlamydospores are uninucleate. He does not mention clamp connections, although his figures suggest strongly that they were present in his material. Hyphae which do not fuse, and therefore remain uninucleate, do not form spores. Seyfert (11) also made some cytological studies and concluded that clamp connections were very abundant on the hyphae inside of the corn plant a considerable time before the formation of chlamydospores.

The writers made numerous observations to ascertain whether the sporidia of M2 would fuse with those of opposite sex. Forms M2 and M3, M2 and M7, and M2 and M4 were grown side by side on potato dextrose agar in petri dishes. In all cases the colonies grew together but no fused sporidia could be found. These forms also were grown together in flasks of dextrose and malt solution. There usually was rather abundant surface growth, but on microscopic examination of this material no evidence of sporidial fusions could be detected. Hanging drops in van Tieghem cells also were inoculated with single forms and with the proper combinations, but no fusions could be observed. Finally the method described by Bauch (2) was used. Sporidia of the individual forms were first transferred separately into test tubes containing a 0.01 per cent malt solution. The tubes were then shaken and approximately 2 cc. of each poured into a petri dish. Bauch found that the sporidia of different sexual strains of *Ustilago violacea* copulated abundantly within two days under these conditions. The writers could find no evidence that the sporidia of *U. zeae* copulated, although more observations will be made.

A histological study was made to ascertain whether clamp connections were formed in corn plants inoculated with single physiologic forms and in those inoculated with two. It was impossible to find any evidence of clamp connections in the tissues of corn plants inoculated with single forms or with combinations of forms which failed to cause the formation of galls. However, hyphal fusions and numerous clamps were observed in the tissues inoculated with forms M2 and M3, M2 and M4, and M2 and M7.

CONCLUSIONS

Ustilago zeae evidently is heterothallic. In the present investigations it was shown that certain physiologic forms alone would not cause infection, but when used in combination with M2 they caused infection readily. The question naturally arises as to whether these so-called physiologic forms therefore actually are physiologic forms or merely different sexual strains. The writers are of the opinion that it is justifiable to consider them as physiologic forms because they differ from one another in appearance and also in

their biochemical reactions, as is indicated by their behavior when grown on standard bacteriological media. As they differ from one another in many characters other than sex, it seems reasonable to consider them as physiologic forms in which the sporidia are all of one sex.

Many physiologic forms with which the writers made inoculations caused infection alone. Hence it seems likely that they comprise two morphologically similar sexual strains. Experiments are now under way to ascertain whether this is true.

The results suggest that many of the physiologic forms of *U. zea* have arisen by hybridization, and it seems probable also that new forms are continually arising by the same process. Whether the mutations previously described by the writers can be explained on the basis of segregation remains to be seen. Some of the apparently unisexual strains used in these experiments produce sectors under various conditions. Of course these strains may comprise several physiologic forms, all of the same sex. However, there is good evidence that true mutants appear. This phase of the problem is being investigated in detail and the results will be published later.

SUMMARY

1. *Ustilago zea* apparently is heterothallic. The fusion of two strains of opposite sex seems to be prerequisite to the formation of chlamydospores in the host plant.

2. When corn is inoculated with certain physiologic forms of *Ustilago zea* alone, no smut galls are formed, but when inoculated with these same forms in combination with another form, presumably of opposite sex, smut galls are formed on a large percentage of plants.

3. So far, evidence has been obtained for the existence of only two sexes.

4. Some physiologic forms alone cause gall formation, suggesting that these forms comprise morphologically isogamous but functionally heterogamous strains.

5. No evidence was obtained that the sporidia of strains of opposite sex fused in artificial culture. However, hyphal fusions and numerous clamp connections were observed in plants which had been inoculated with two strains of opposite sex but not in those which were inoculated with unisexual strains alone, nor in those inoculated with combinations of strains of the same sex.

6. It is suggested that new physiologic forms are being produced by hybridization and consequent segregation.

7. The production of sectors by certain unisexual strains suggests that new forms are arising by mutation also.

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PHYTOPATHOLOGICAL NOTES

Effects of fungous extracts upon the initiation and growth of the perithecia of Venturia inaequalis (Cke.) Wint. in pure culture.—The stimulatory effect of fungous extract upon the production of perithecia by *Thielavia basicola* Zopf has been reported by Miss McCormick.¹ In some recent work the present writer observed a similar stimulatory effect when certain fungi occurred as contaminants in petri-dish cultures of *Venturia inaequalis*. The perithecia of *V. inaequalis* were more abundant and of greater size in a zone near the periphery of the colony of the contaminant. A *Penicillium* was grown in pure culture on oatmeal agar. After two weeks the agar containing this fungus was macerated in clean white sand and a water extract made. This extract was forced through a Berkefeld filter, and the filtrate was found to be sterile when small amounts of it were plated out. Five petri-dish cultures of *V. inaequalis* on oatmeal agar, in which no perithecia had appeared, were treated with 1 cc. each of this extract; five cultures were treated with 1 cc. each of the same extract after it had been autoclaved at 12 pounds pressure for 20 minutes; and five cultures were treated with 1 cc. each of sterile distilled water. The liquid was spread over the agar by tipping the petri dishes from side to side. After keeping these cultures at 16° C. for one week, data were taken on the abundance of the perithecia and on their diameters. Perithecia were counted in 40 microscopic fields selected from each plate in areas where the fungus occurred in abundance (one microscopic field was 2.5 sq. mm. in area). Measurements were made of the diameters of 50 perithecia in each plate, when that number could be found. The results appear in table 1.

TABLE 1.—*The effect on number and size of perithecia when Penicillium extracts are added to cultures of Venturia inaequalis*

Liquid added to cultures	Perithecia	
	Av. no. per 100 sq. mm.	Av. diam.
Check, 1 cc. sterile water.....	2	37 μ
1 cc. Penicillium extract, autoclaved.....	6	49 μ
1 cc. Penicillium extract, not autoclaved	25	67 μ

Furthermore, there had been a greater vegetative development of the fungus in the cultures treated with the unautoclaved extract. It is noteworthy that there appeared to be a slight stimulation both of perithecial and

¹ McCormick, Florence A. Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. Conn. Agr. Exp. Sta. Bul. 269: 539-554. 1925.

mycelial development in the plates treated with the autoclaved extract.--
E. E. WILSON, *College of Agriculture, Madison, Wisconsin*.

Summer meeting.—The summer meeting of the American Phytopathological Society, under the auspices of the Advisory Board, was held in 1921 in Ohio, August 16–19. Local arrangements were in the hands of a committee composed of H. C. Young, W. G. Stover, Curtis May, Paul E. Tilford, and R. C. Thomas. The meeting took the form of a tour of northern portions of the state for the purpose of studying, primarily, diseases of fruits and vegetables. Preceding the tour, an inspection of the laboratories, greenhouses, and Experiment Station farm was made. Registration of 70 in attendance showed good representation from all sections east of the Mississippi River and from Canada. Variety marked the meeting: a large number of crops and diseases was observed; types of commercial field production were visited; and any possible monotony which might have arisen from an extended auto tour was broken by a boat trip to Kelley's Island and Put-in-Bay.

One of the striking features of the meeting was the results noted in plots of oats which had been treated in various ways for smut. Two new dusts, with formaldehyde and iodine respectively as the base, gave remarkably complete control over heavily smutted checks. Further announcement of this work is to be made in *Science* at an early date.

Potato spraying and dusting experiments were inspected both in plots at Wooster and on a large farm near Smithville where there were 250 acres of potatoes. Visitors availed themselves of the ample opportunity afforded in the field for full discussion of the several aspects of potato disease control.

Other striking and interesting studies included plots of Michigan resistant celery, onion pink root, raspberry virus troubles, winter injury on peaches, *Bacterium pruni* in relation to fertilizers, and grape spraying.

The many pleasant features of the meeting can not be enumerated. Breakfast, served in the laboratories at the Experiment Station, made possible the beginning of good fellowship which prevailed throughout the tour. The Celery Growers' Association at Celeryville gave a supper which promoted mutual acquaintanceship between the growers and pathologists. Impromptu talks, by Mr. Nelson on celery root rot and Michigan resistant celery, by Dr. Newhall on "seeders," and by Dr. Chupp on celery leaf diseases, were plainly impressive. The growers displayed keen interest in the questions raised by the speakers, while the younger pathologists in attendance were given a demonstration of good methods of extension presentation.

According to the visitors, credit for the success of the excellent program, both of fun and business, is due the Ohio pathologists.

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